

HU: MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAAPPGASAYGSL
MO: MGSDVRDLNALLPAVSSLGGGGCGLPVSGAAQWAPVLDFAAPPGASAYGSL

HU: GGPAPPPAPPPPPPPPHSF1KQEP SWGGAEPHEEQCLSAFTVHFSQFTGTAG
MO: GGPAPPPAPPPPPPPPHSF1KQEP SWGGAEPHEEQCLSAFTLHFSQFTGTAG

HU: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS
MO: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU: YGHTPSHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVGCHTPDSCTG
MO: YGHTPSHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVGCHTPDSCTG

HU: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLAGTLKGVAAGSSSVKWE
MO: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLAGTLKGMAAGSSSVKWE

HU: GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS
MO: GQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS

HU: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR
MO: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU: SDQLKRHQRRTGVPKFQCKTCQRKFSRSDHLKTHTRHTGKTSEKPFSCR
MO: SDQLKRHQRRTGVPKFQCKTCQRKFSRSDHLKTHTRHTGKTSEKPFSCR

HU: WPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
MO: WHSCQKKFARSDELVRHHNMHQRNMTKLHVAL

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

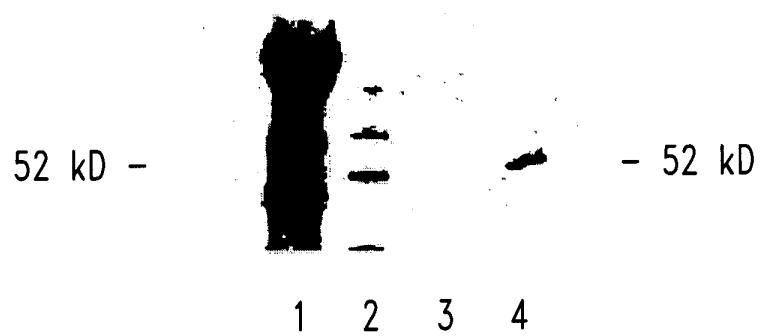


Fig. 2

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

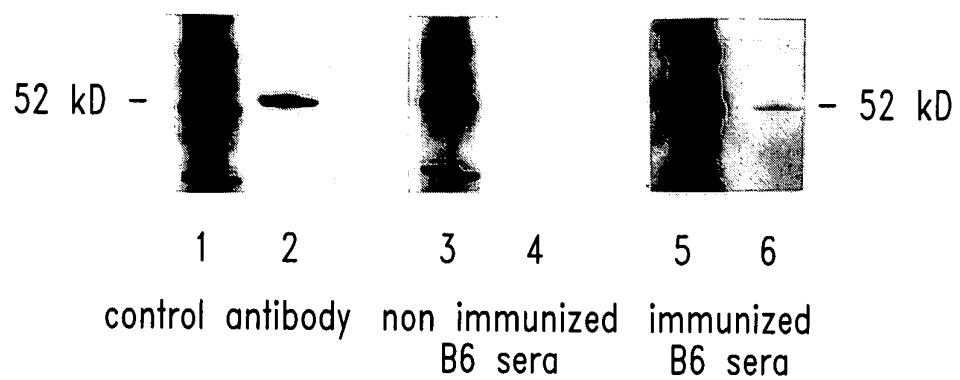


Fig. 3

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

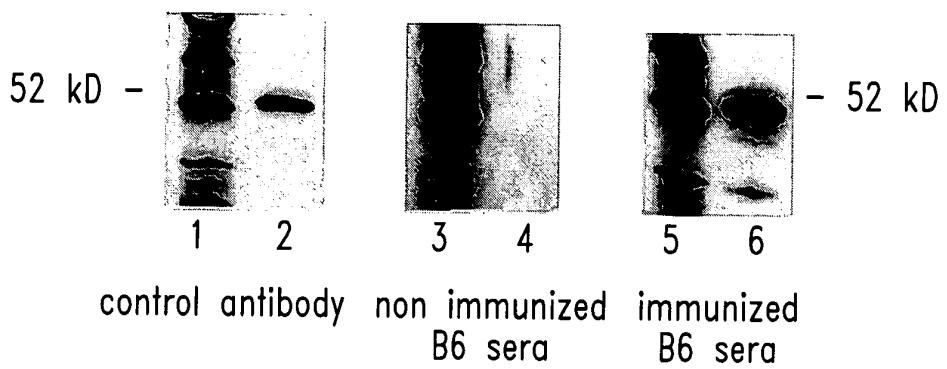


Fig. 4

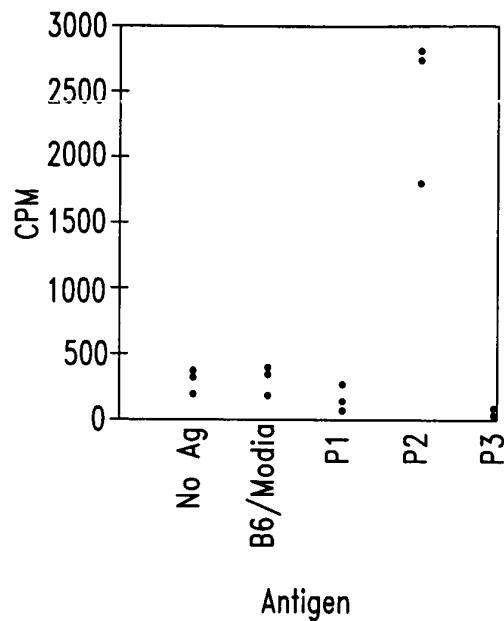


Fig. 5A

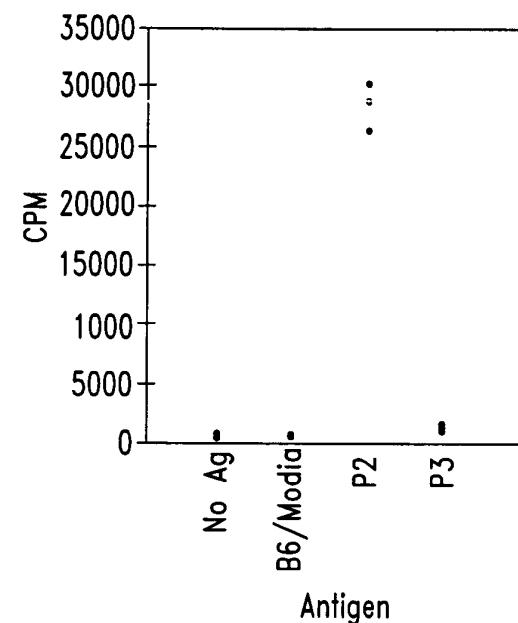


Fig. 5B

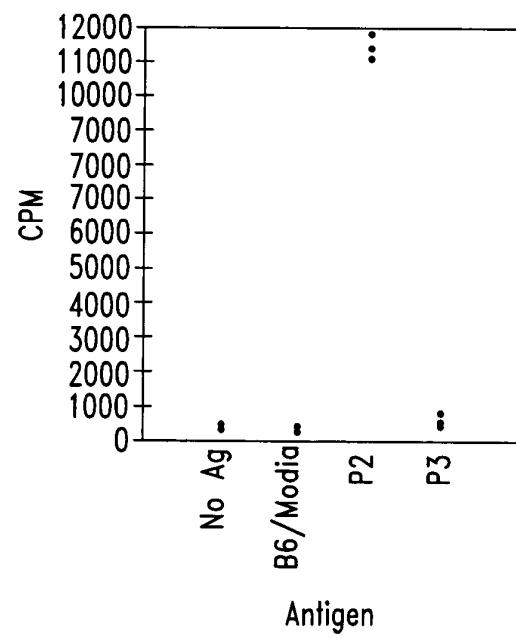


Fig. 5C

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

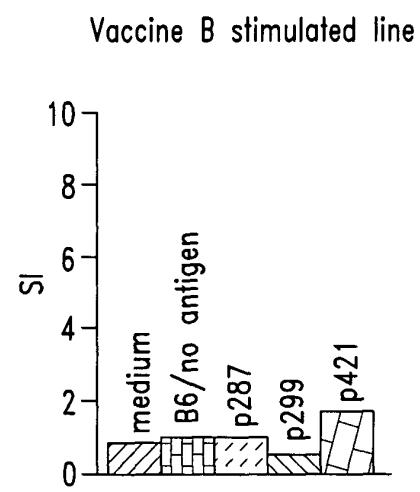
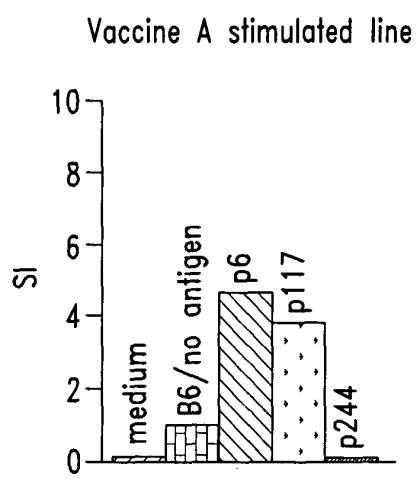
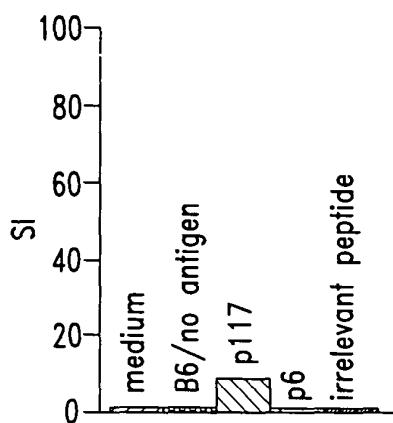


Fig. 6A

Fig. 6B

p117-139 stimulated line



p117-139 stimulated clone

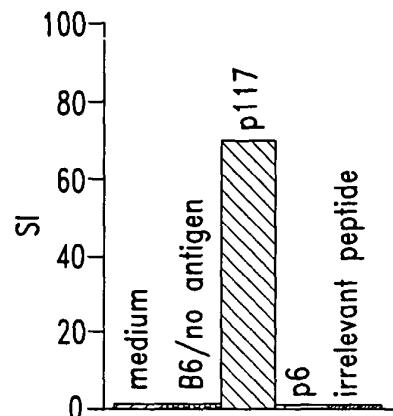
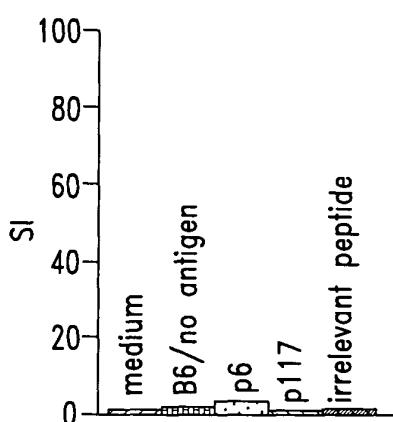


Fig. 7A

Fig. 7B

p6-22 stimulated line



p6-22 stimulated clone

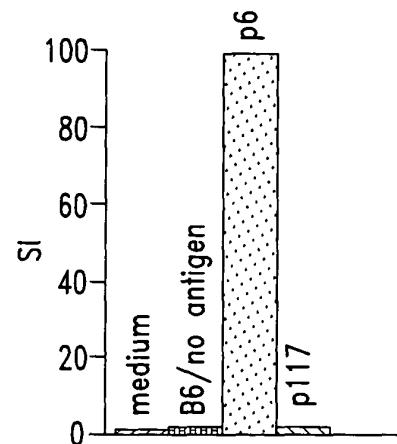


Fig. 7C

Fig. 7D

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
MGSDVRLNALLPAVPSLGGGGCALPVSGAAQWAPVLDAPPAGSAYGSLGGPAPPPAPPPPPPPHSFIQEE
.....AAAAAAA.....AAAAA.....AAAAAAA.....
.....RRRR.....
.....
.....
80 85 90 95 100 105 110 115 120 125 130 135 140 145 150
PSWGGAEPEEQQCLSAFTVHFSQFTGTAGACRYGPFGPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYS
.....AAA.....AAAA.....AAA.....AAAAA.....
.....RRRR.....RRRRR.....
.....DDDDDDDDDD.....
.....
155 160 165 170 175 180 185 190 195 200 205 210 215 220 225
TVTFDGTPSYGHTPSHAAQFPNHSFKHEDPMGQQGSLGEQQYSPPPVYGCHTPTDSCTGSQALLRTPYSSDN
.....AAAA.....AAAAA.....AA.....
.....RRRR.....
.....DDDDDDDDDDDD.....
.....
230 235 240 245 250 255 260 265 270 275 280 285 290 295 300
LYQMTSQLEMTWNQMNLTGVAAGSSSVKWTGQSNHSTGYESDNHTTPILCGAQYRHTHGVFRGIQDV
AAAAAAA.....AAA.....AAA.....AAAAAAA.....
.....RRRRRRRRR.....RRR.....RRR.....
.....DDDDDDDDDD.....
.....ddddd.....
.....
305 310 315 320 325 330 335 340 345 350 355 360 365 370 375
RRVPGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSRSDQLKRHQR
AAAAA.....AAAAAAA.....AAAAA.....AAAAA.....
.....RRRRR.....RRR.....
.....DDDDDD.....
.....
380 385 390 395 400 405 410 415 420 425 430 435 440 445 450
RHTGVKPFQCKTCQRKFCSRSDHLKTHTRHTGKTSEKPFSCRWPSQKKFARSDELVRHHNMHQRNMTKLQLAL
.....AAAAA.....AA.....AAA.....AAA.....AAA.....AAAAAAA.....AAA.....
.....RRRR.....RRRR.....
.....ddddd.....

Fig. 8A

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY
Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
MGSDVRDLNALLPAVSSLGGGGCGLPVSGAAQWAPVLDFAAPPGASAYGSLGGPAPPPPPPPPHSFIKQE
.....AAAAAAA.....AAAAA.....AAAAAAA.....
.....RRRR.....

80 85 90 95 100 105 110 115 120 125 130 135 140 145 150
PSWGGAEPEHIEQCLSAFTLHFSGQFTGTAGACRYGPFGPPPSQASSGQARMFPNAPYLPSCLESOPTIRNQGYS
.....AAAA.....AAA.....AAAAA.....
.....RRRR.....RRRRR.....
.....DDDDDDDD.....

155 160 165 170 175 180 185 190 195 200 205 210 215 220 225
TVTFDGAPSYGHTPSHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPDSCTGSQALLRTPYSSDN
.....AAAA.....AAAAA.....AA
.....RRRR.....
.....DDDDDDDDDDDDDD.....

230 235 240 245 250 255 260 265 270 275 280 285 290 295 300
LYQMTSQLCEMTWNQMLGATLKGMAGSSSVKWTGQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDV
.....AAAAA.....AA.AA.....AAAAAAA.....
.....RRRRRRRRR.....RRRR.....RRRR.....
.....DDDDDDDDDD.....
.....ddddd.....

305 310 315 320 325 330 335 340 345 350 355 360 365 370 375
RRVSGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQ
.....AAAAA.....AAAAAAA.....AAAA.....AAAAAAA.....
.....RRRR.....RRRR.....
.....DDDDDDDDDD.....

380 385 390 395 400 405 410 415 420 425 430 435 440 445 450
RHTGVKPFQCKTCQRKFCSRSDLKTHTRHTGKTSEKPFSCRWHSCQKKFARSDELVRHHNMHQRNMTKLHVAL
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.....RRRR.....RRRR.....
.....ddddd.....

Fig. 8B

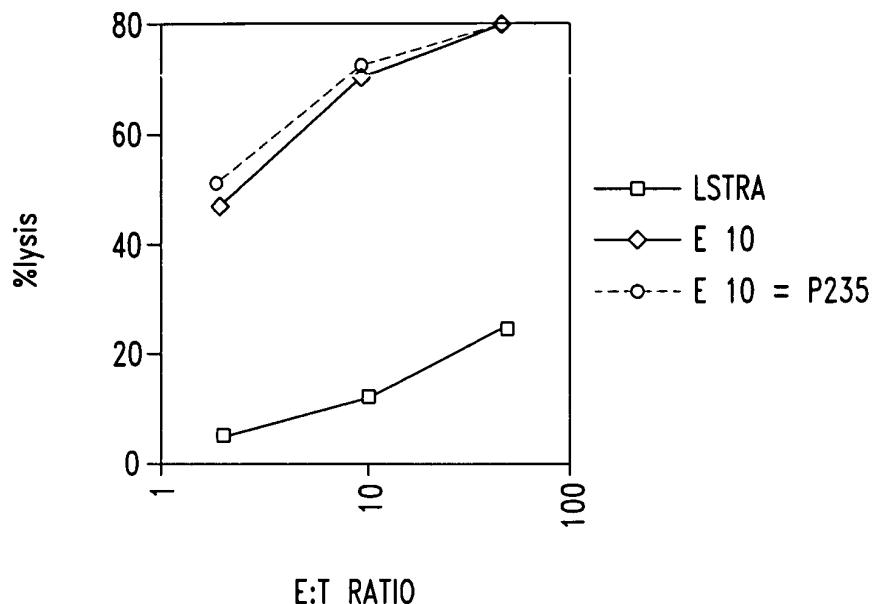


Fig. 9A

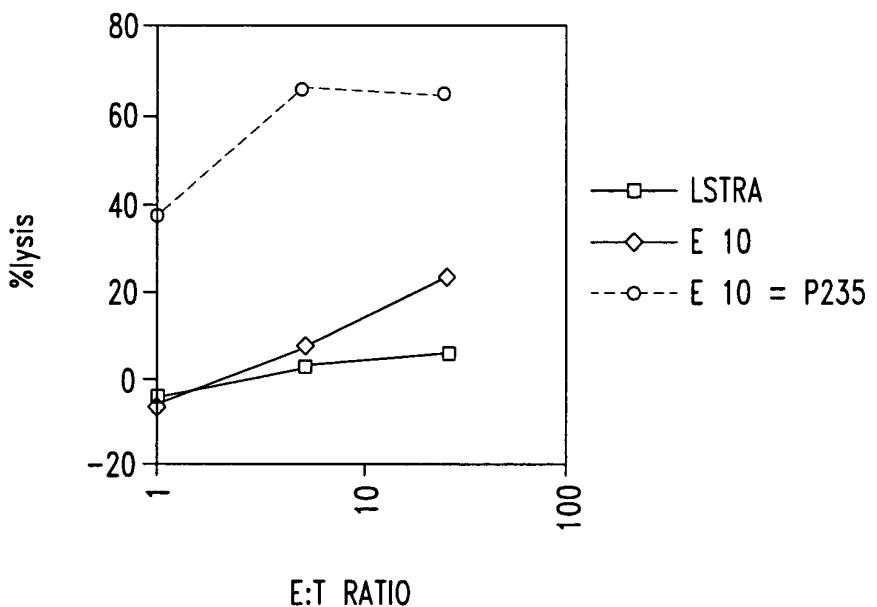


Fig. 9B

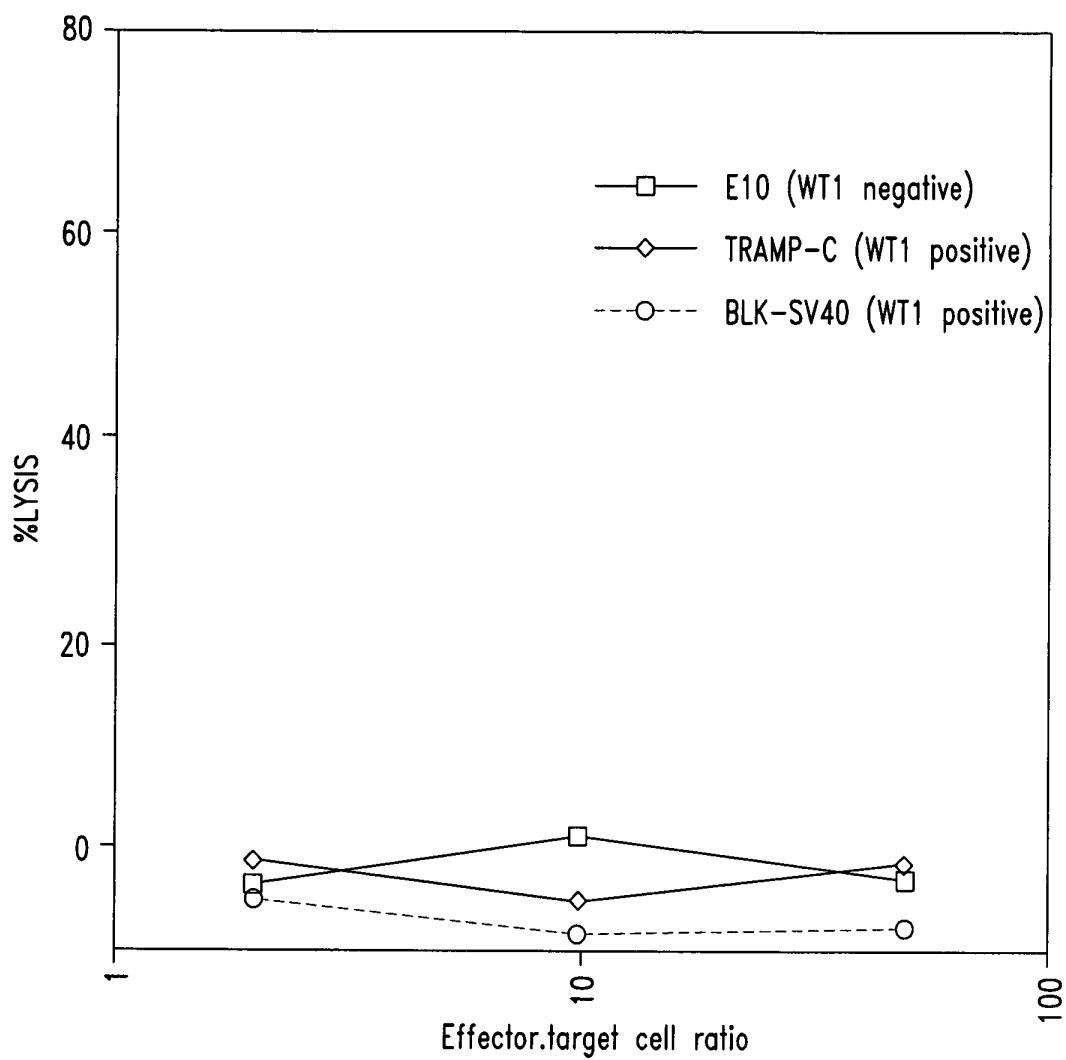


Fig. 10A

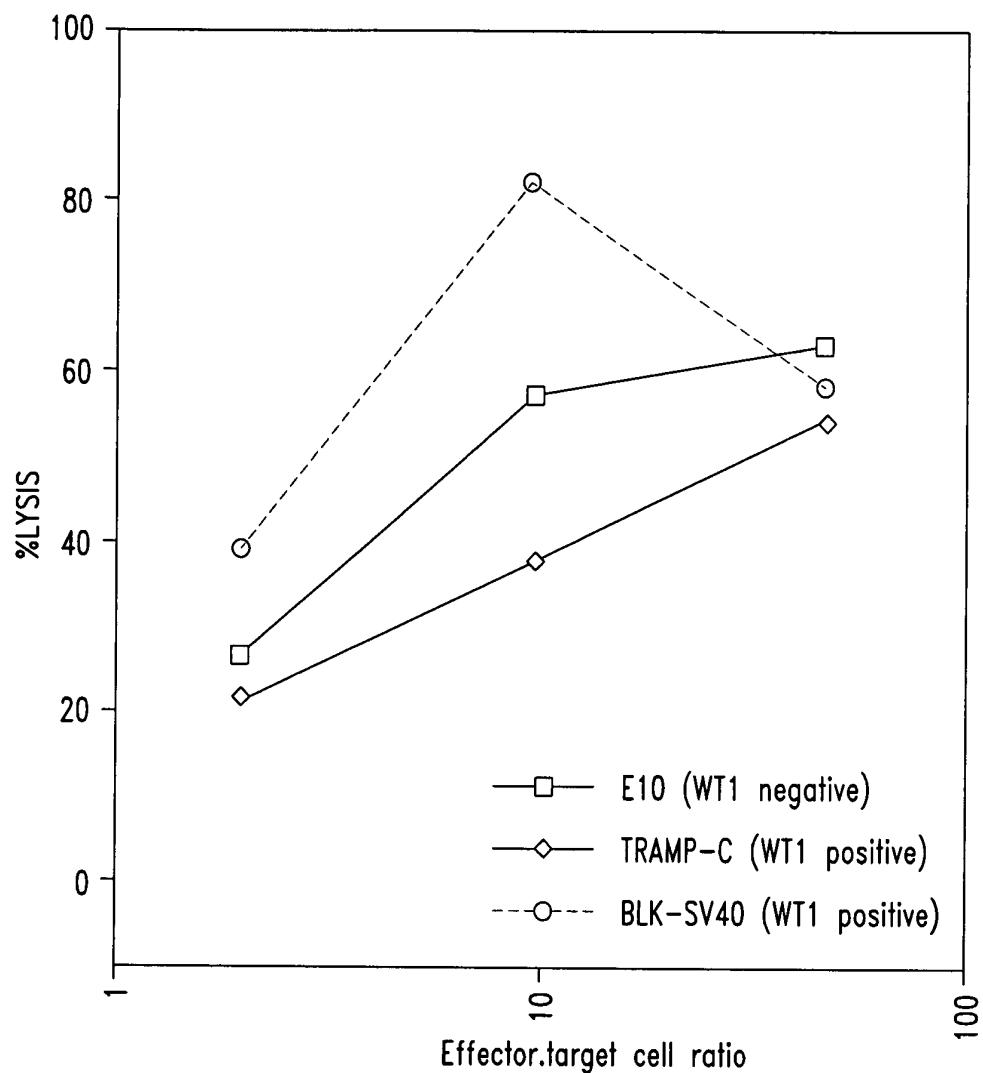


Fig. 10B

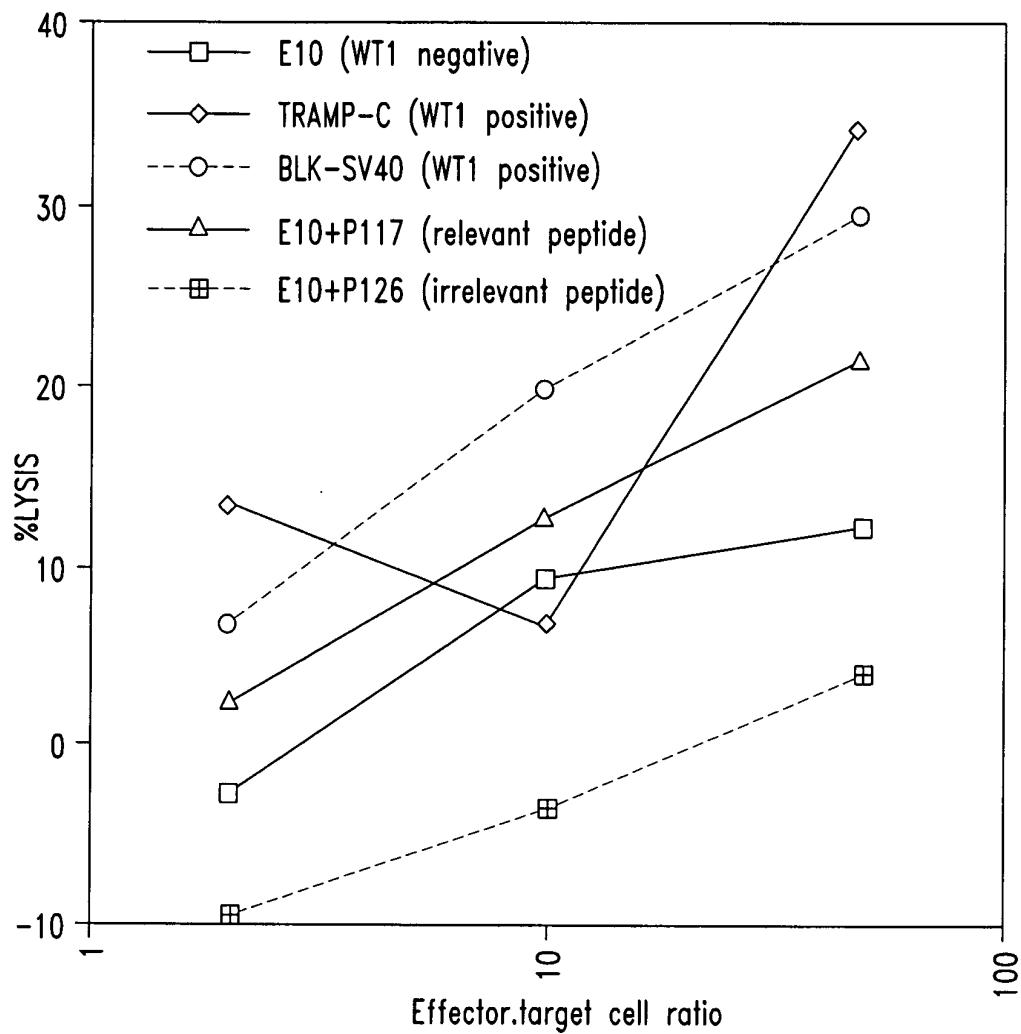


Fig. 10C

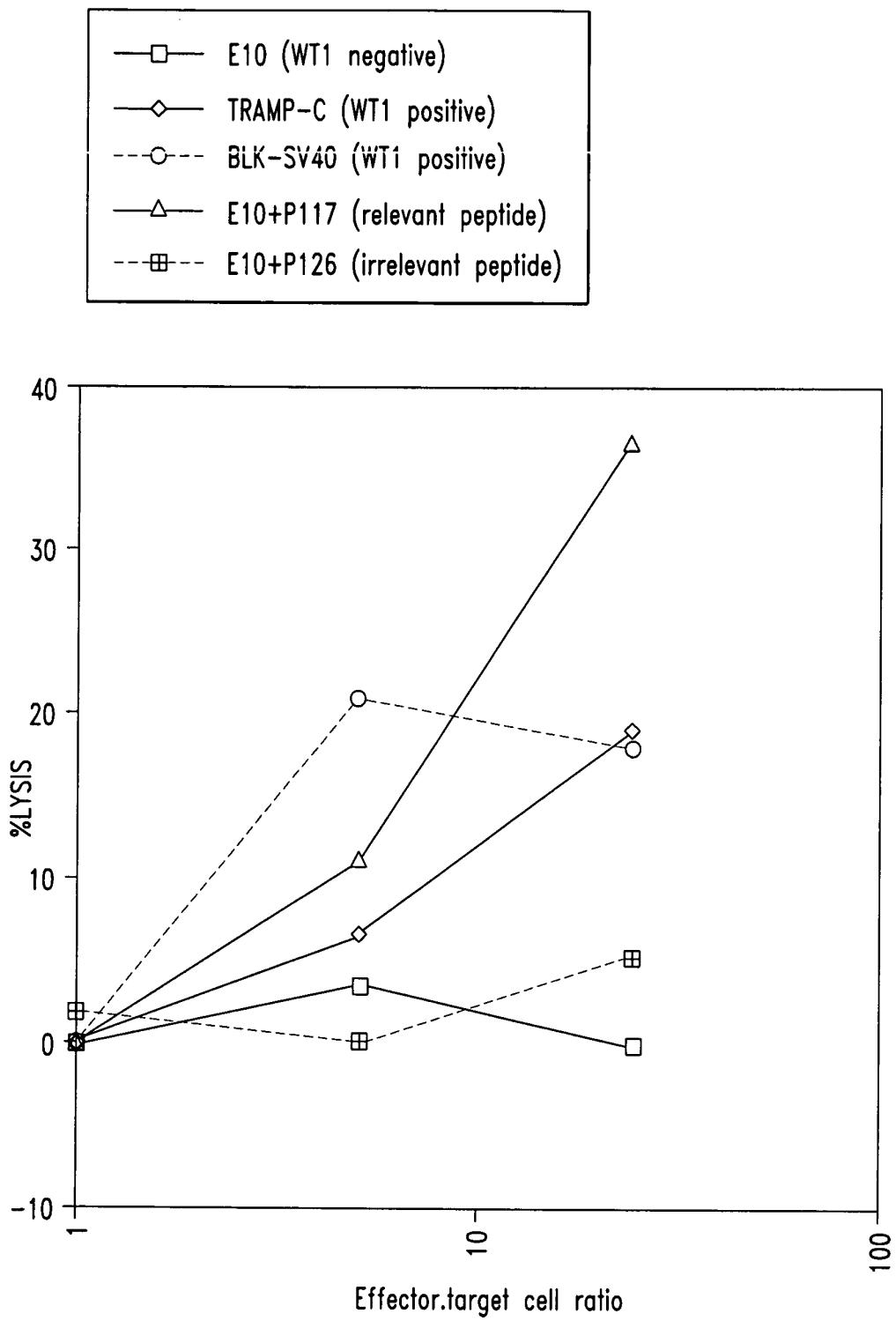


Fig. 10D

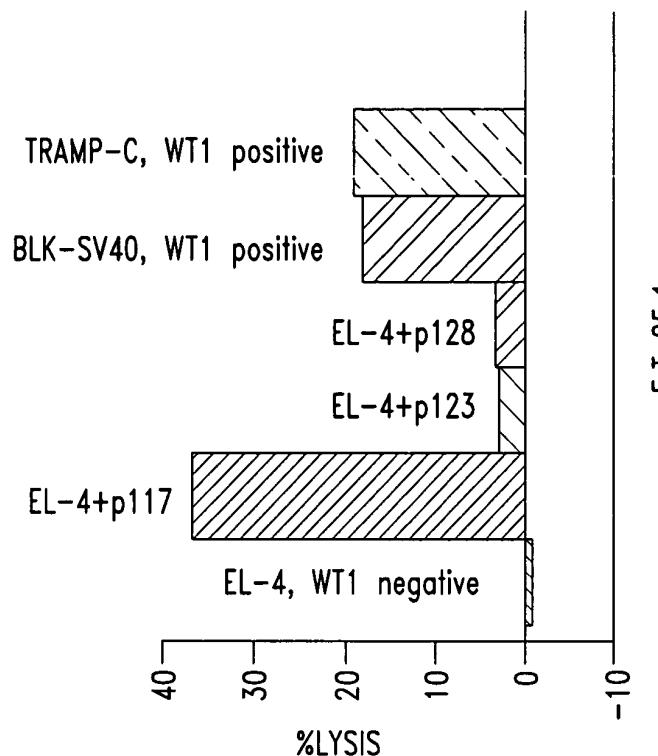


Fig. 11B

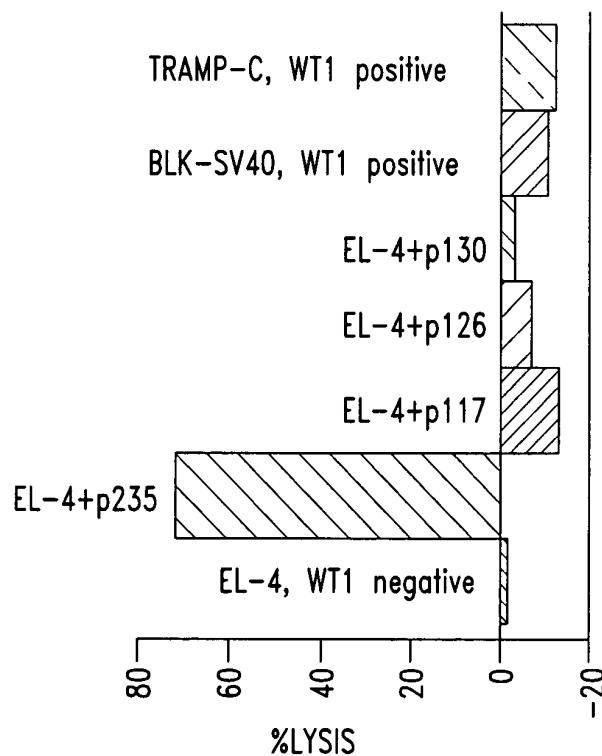


Fig. 11A

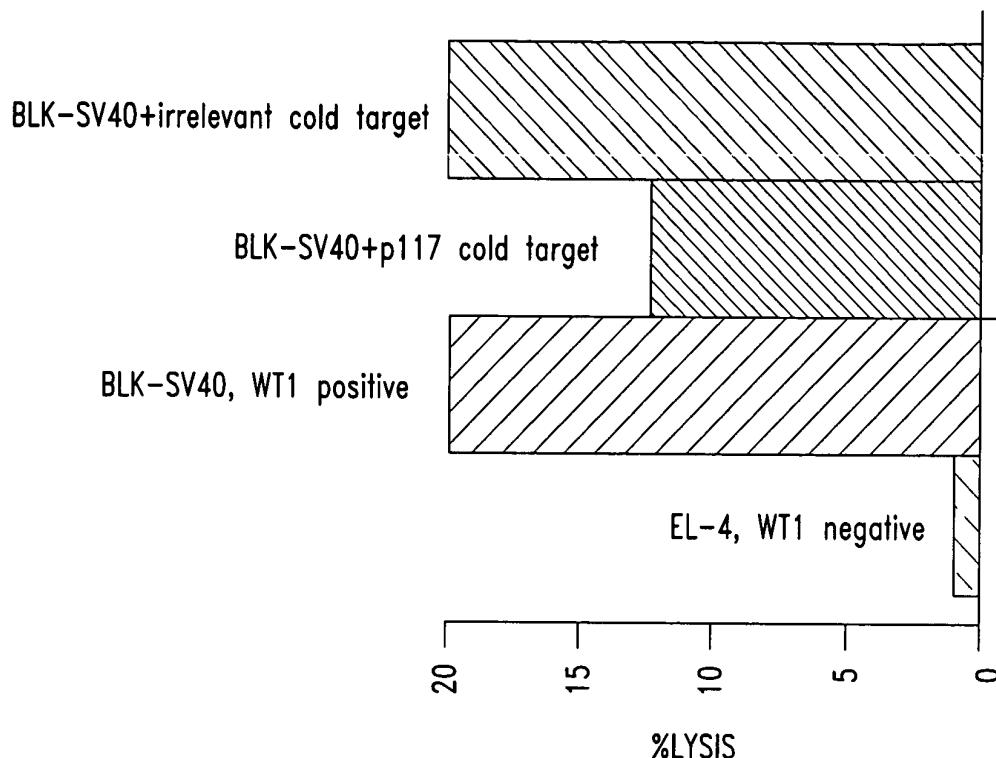


Fig. 12B

E:T 25:1

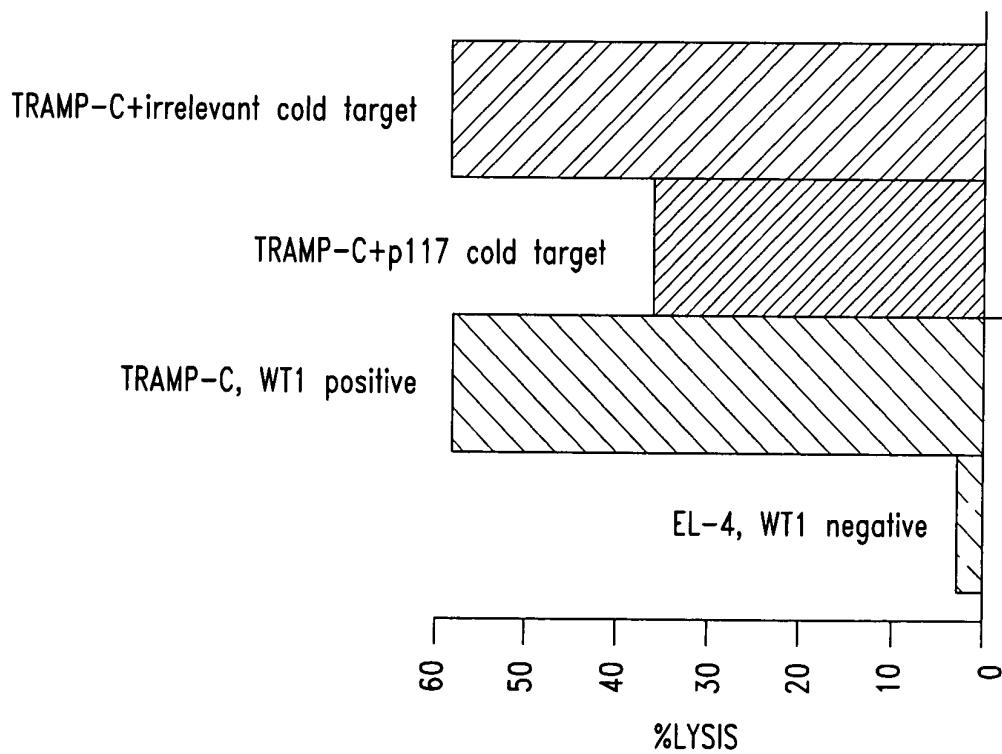


Fig. 12A

E:T 25:1

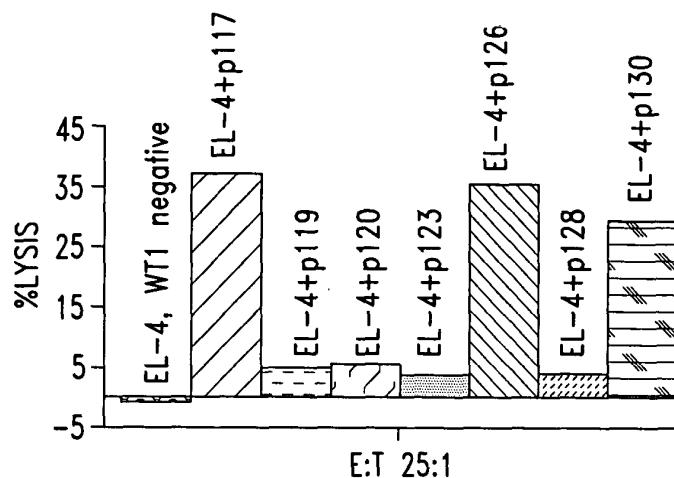


Fig. 13A

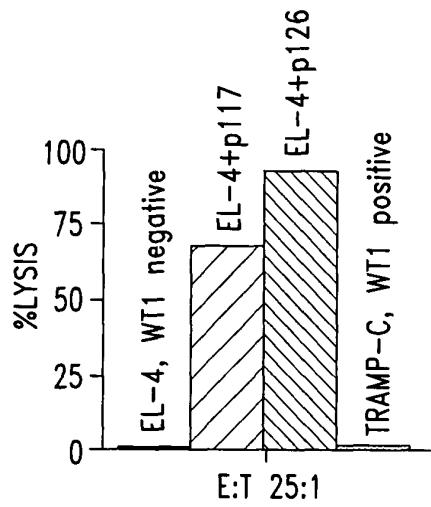


Fig. 13B

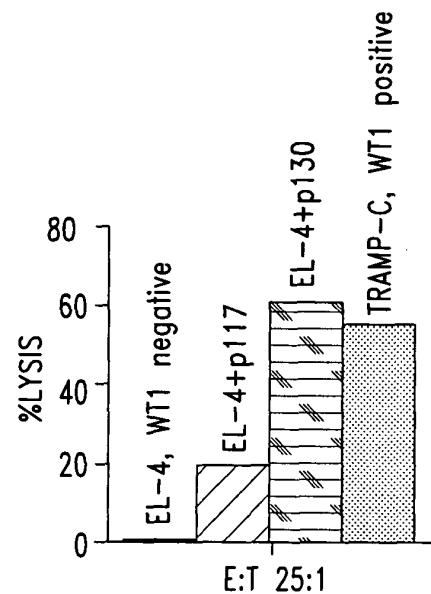


Fig. 13C

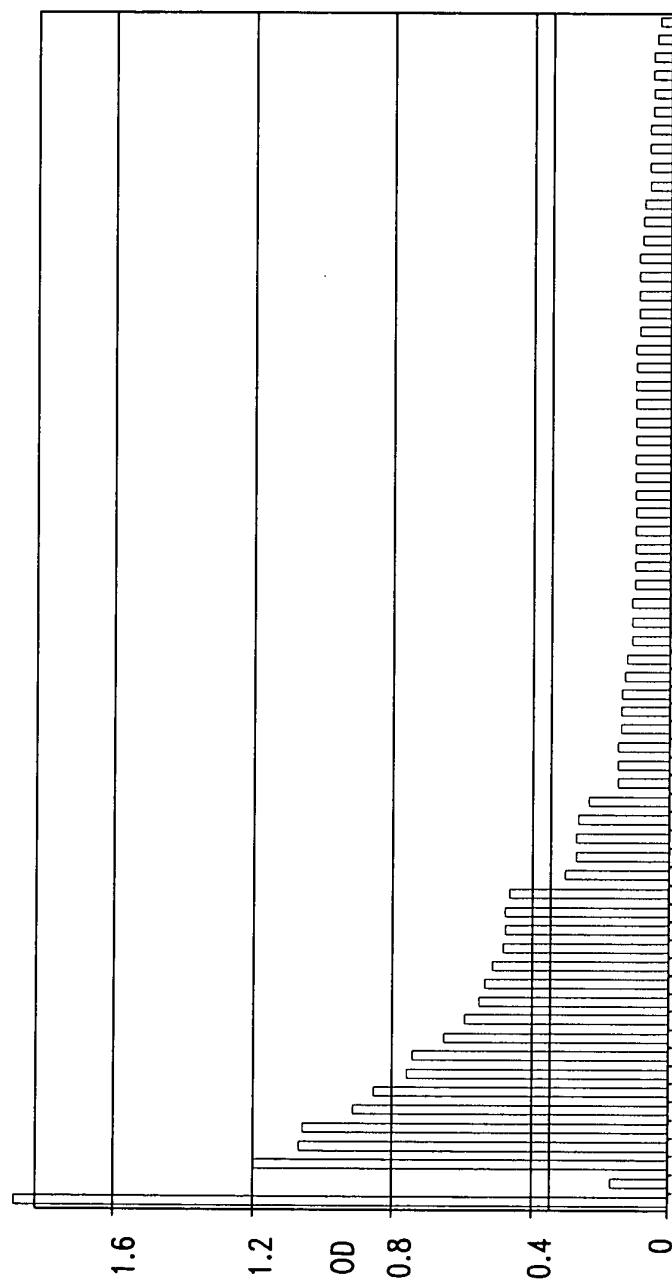


Fig. 14

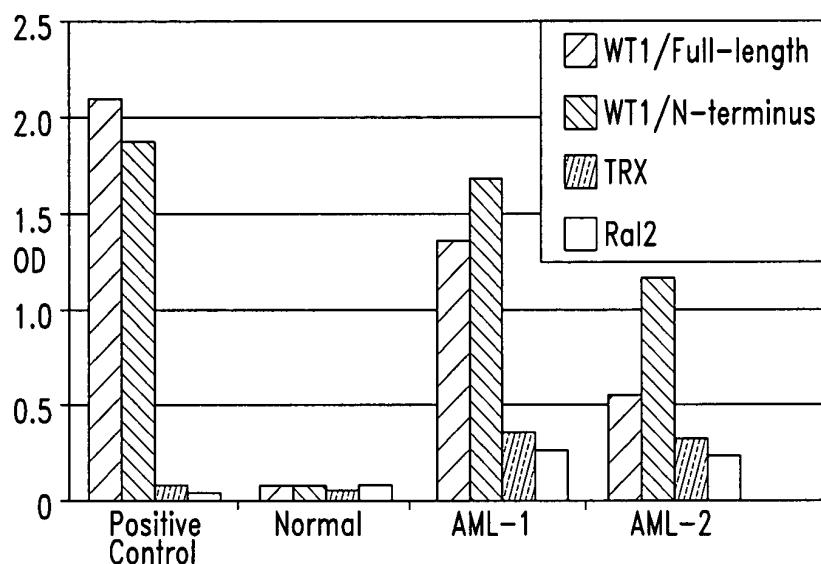


Fig. 15

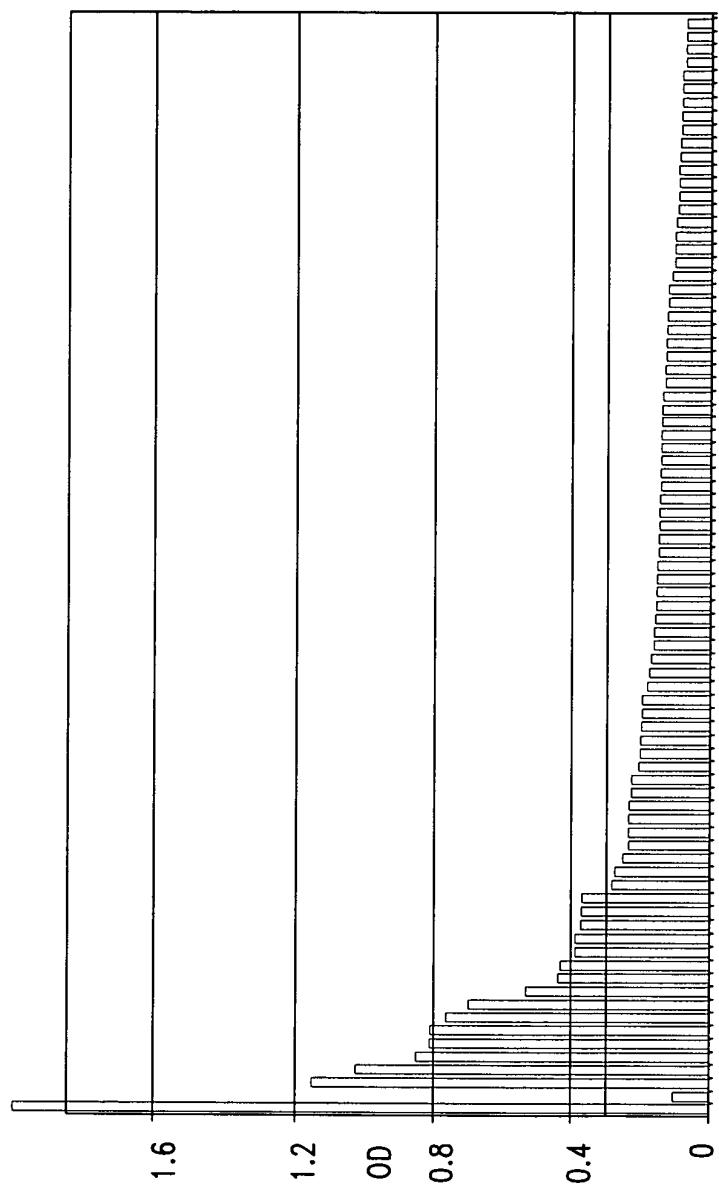


Fig. 16

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12

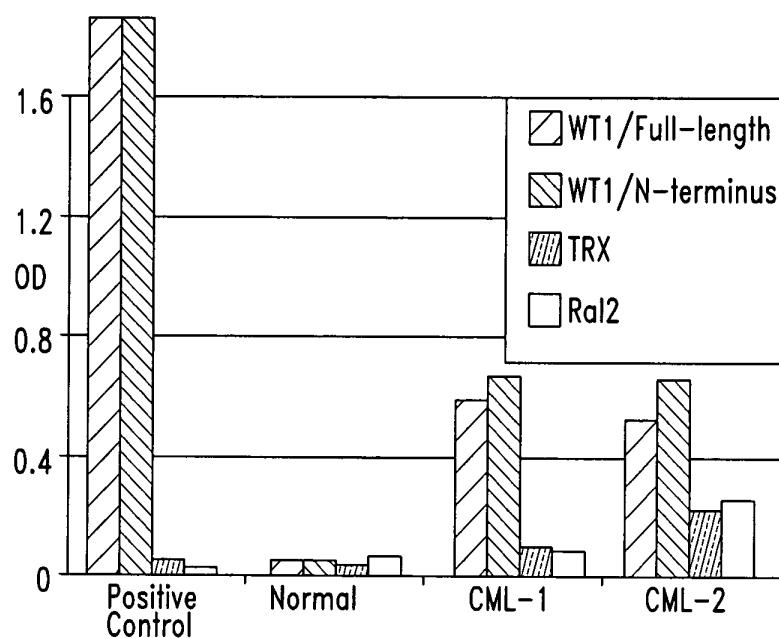


Fig. 17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

<u>NAME</u>	<u>Recombinant Protein</u>	<u>WT1 Amino Acid Position</u>	<u>Molecular Weight</u>
WT1 / full-length	Rat2-WT1 full length fusion protein	aa 1-449	85kDa
WT1 / N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1 / C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

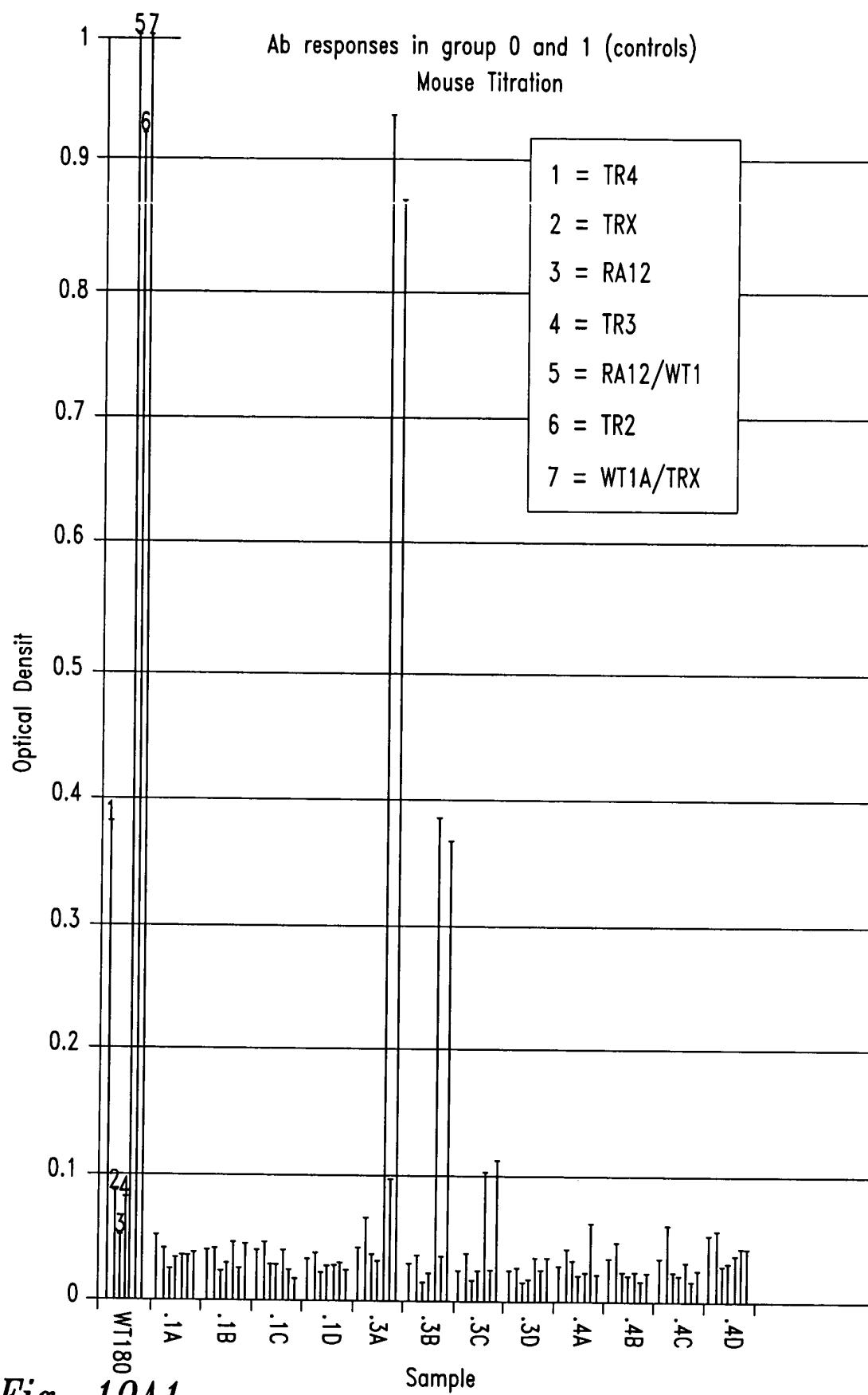
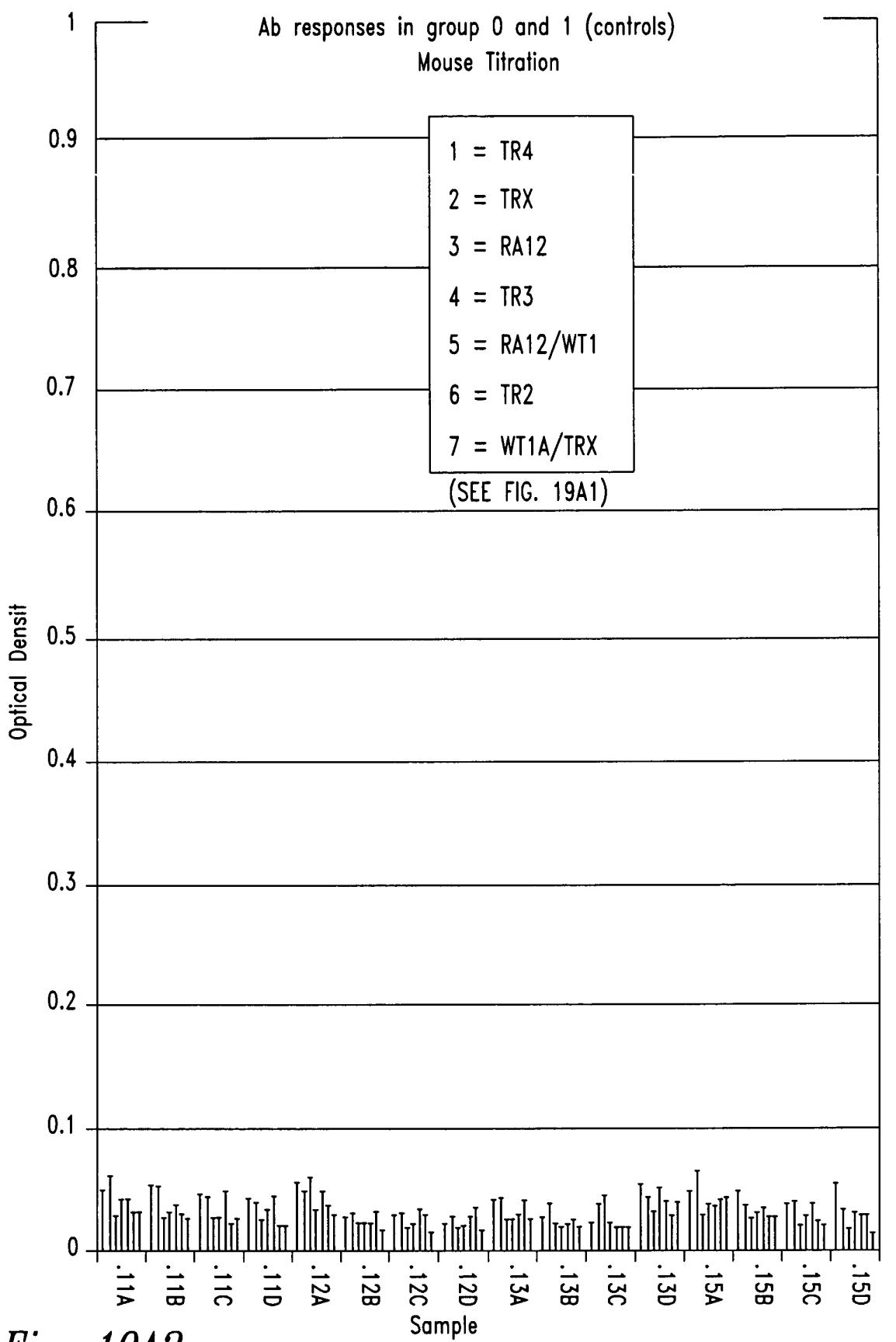


Fig. 19A1 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



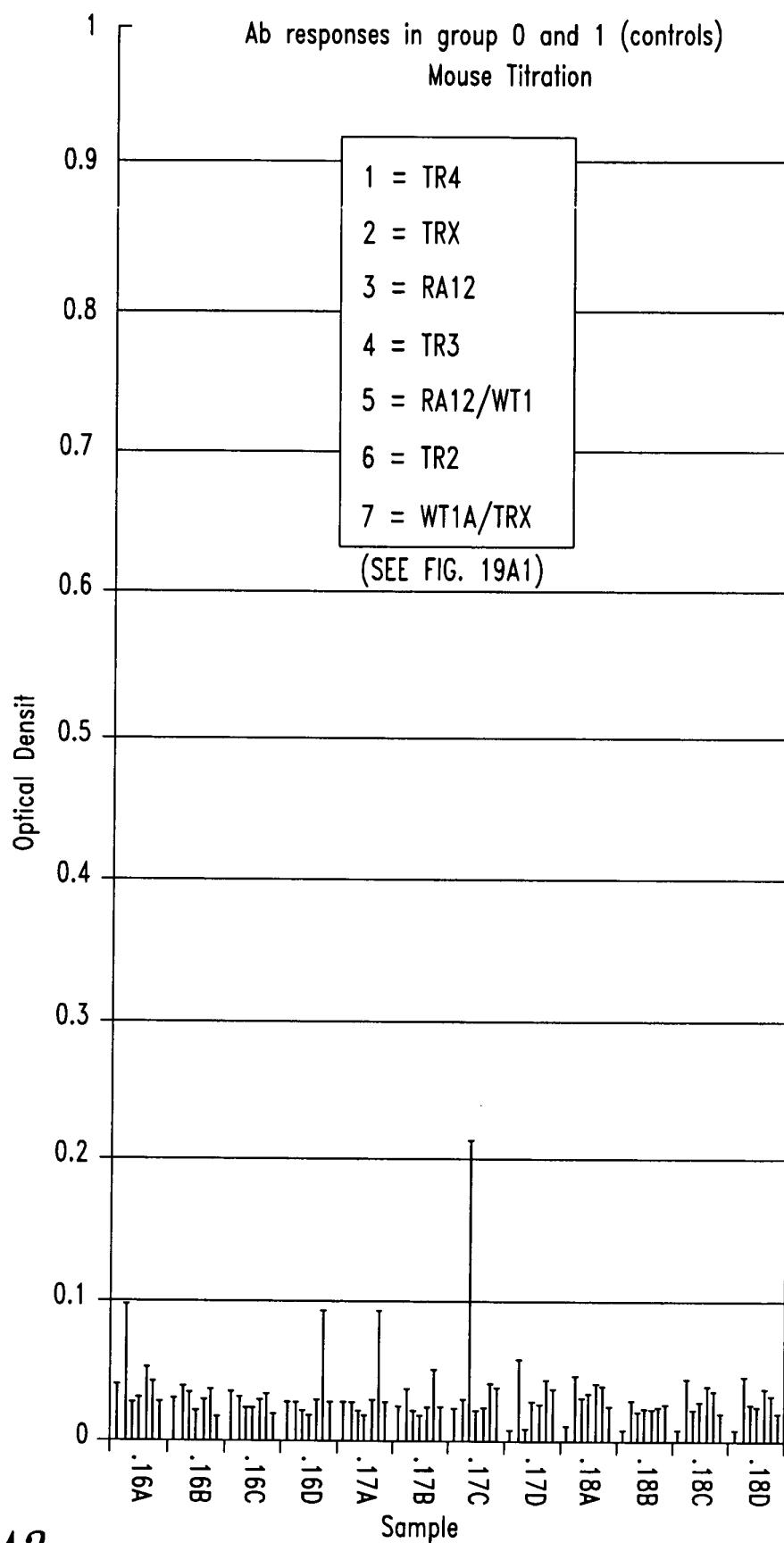
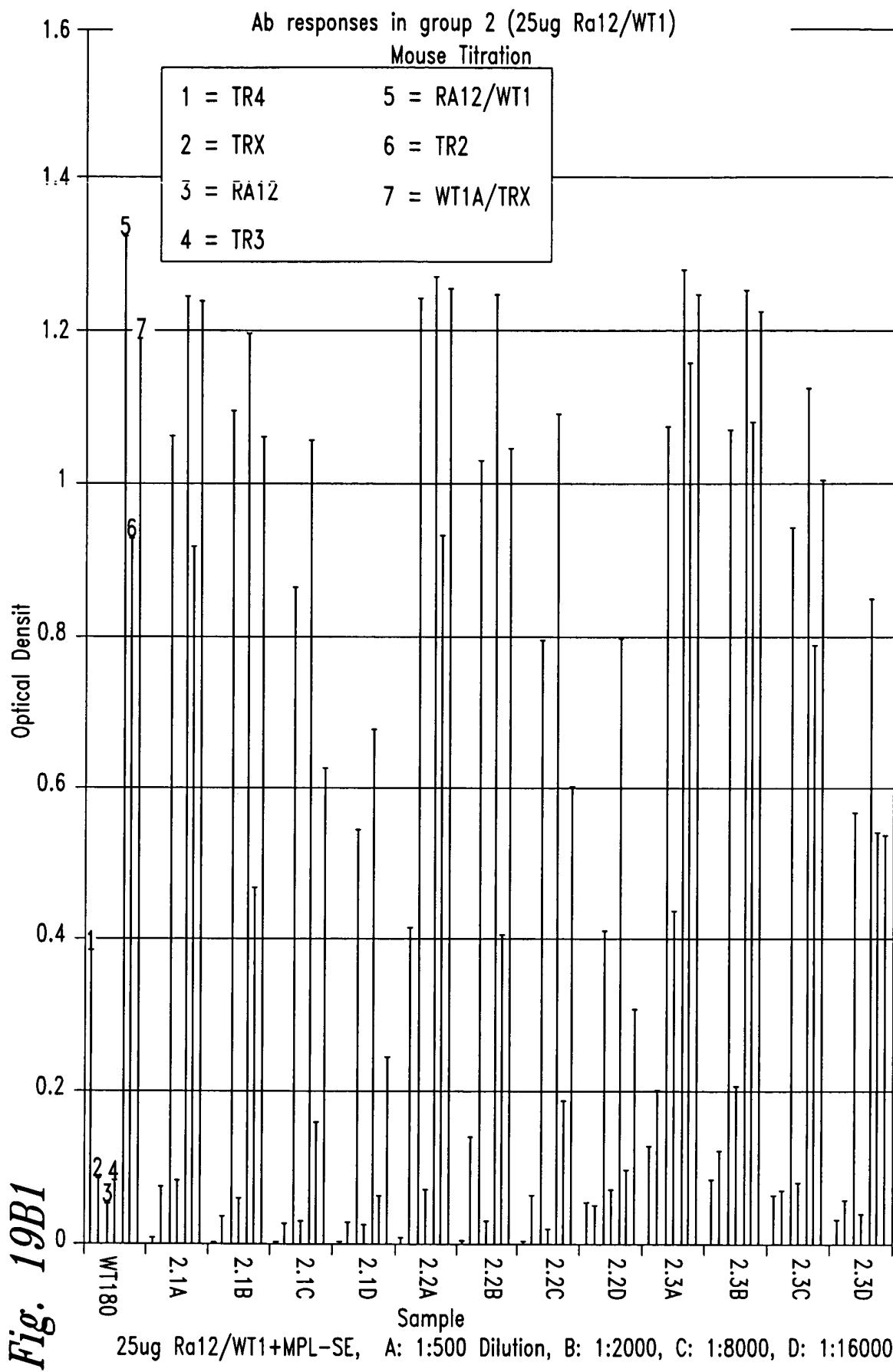
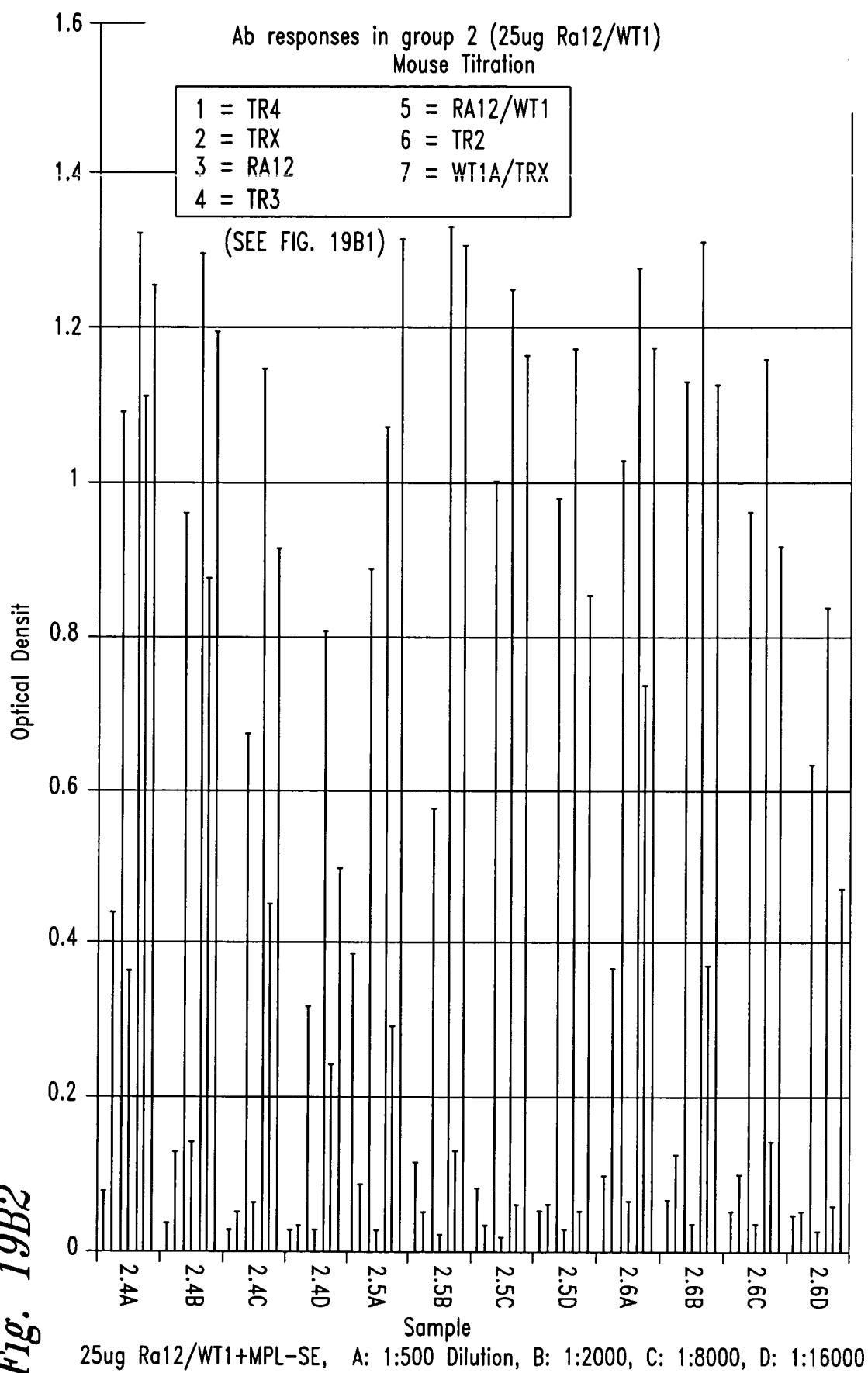


Fig. 19A3 Control groups. A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000





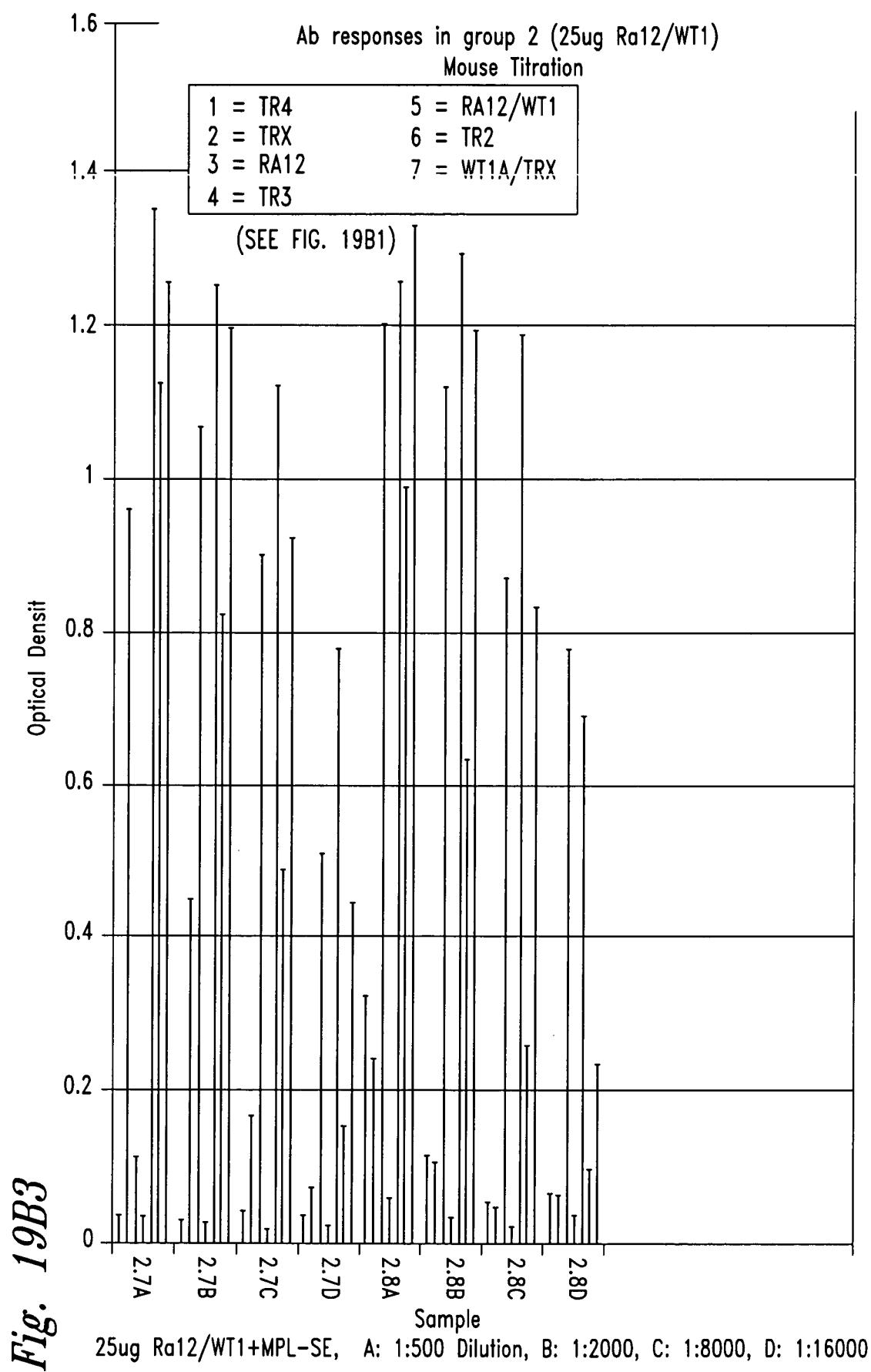
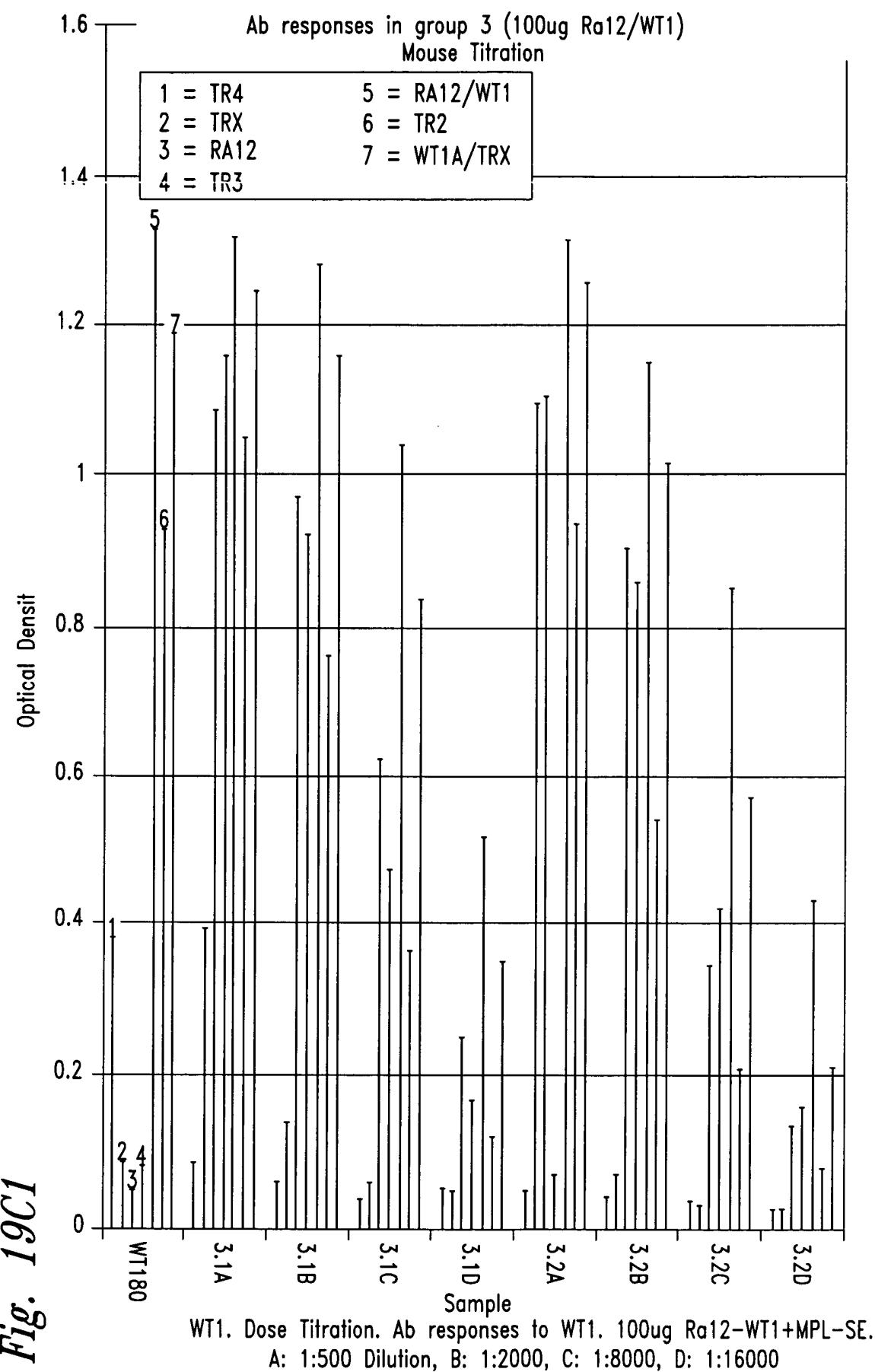
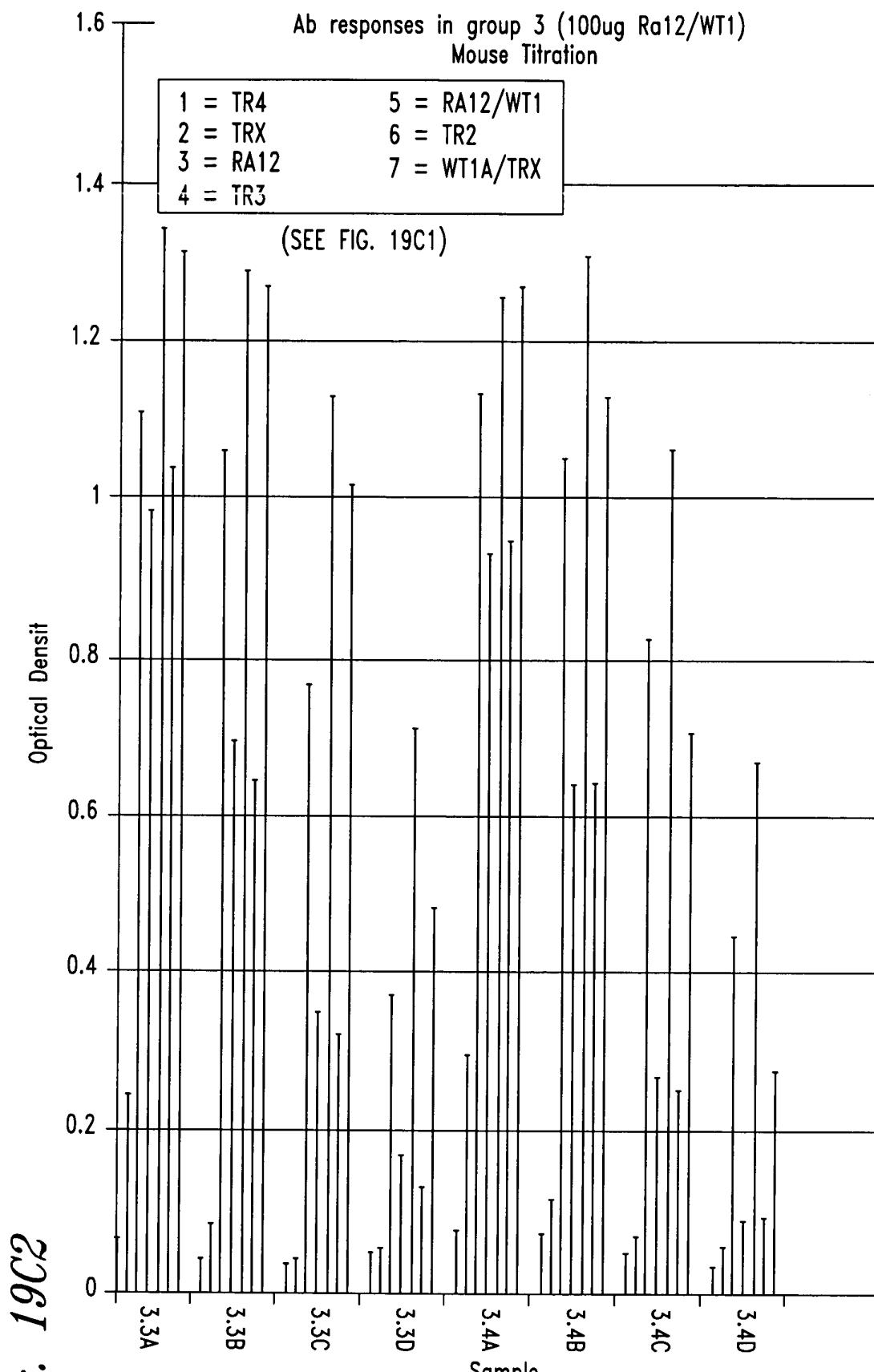


Fig. 19B3





WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

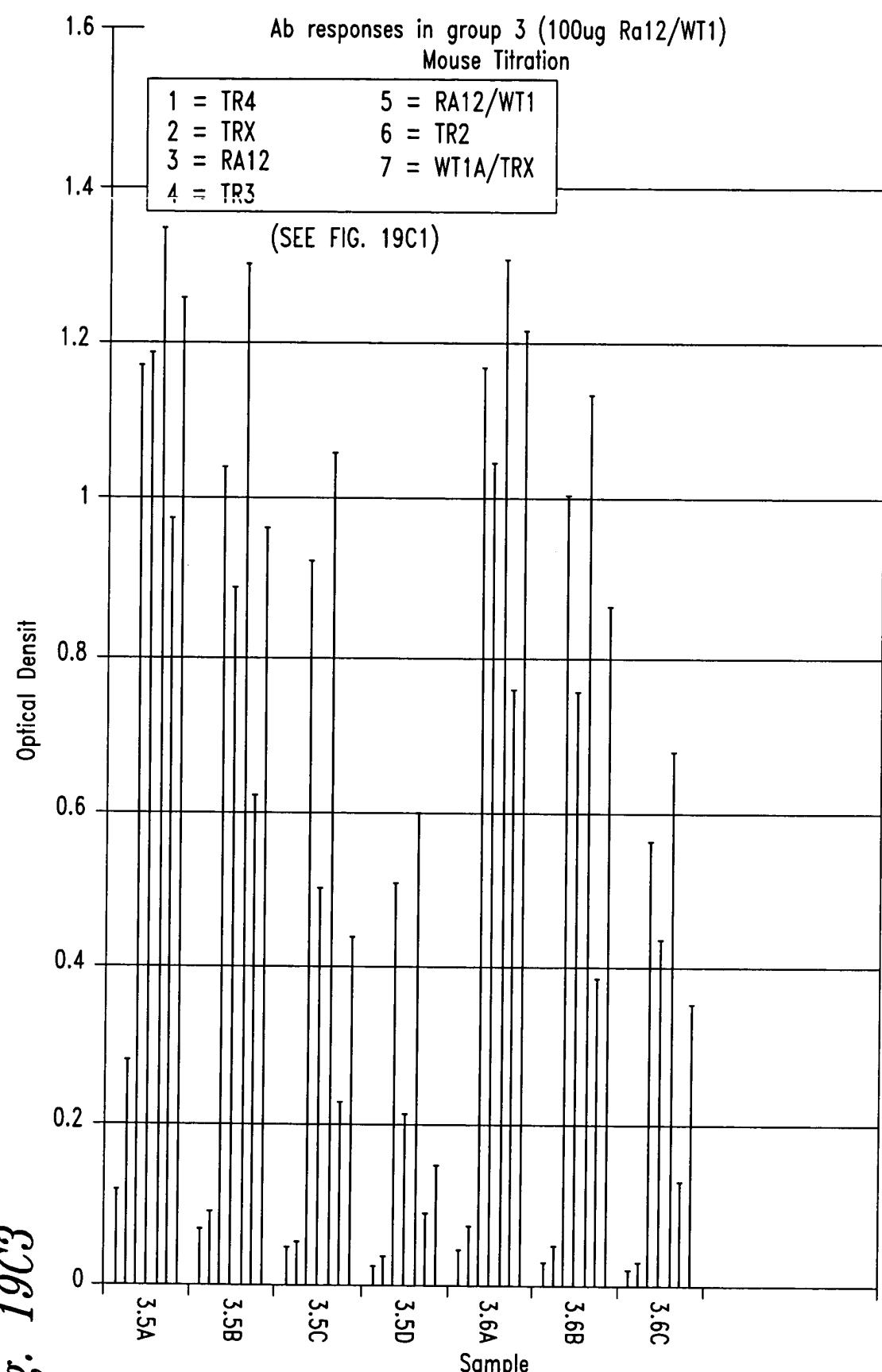
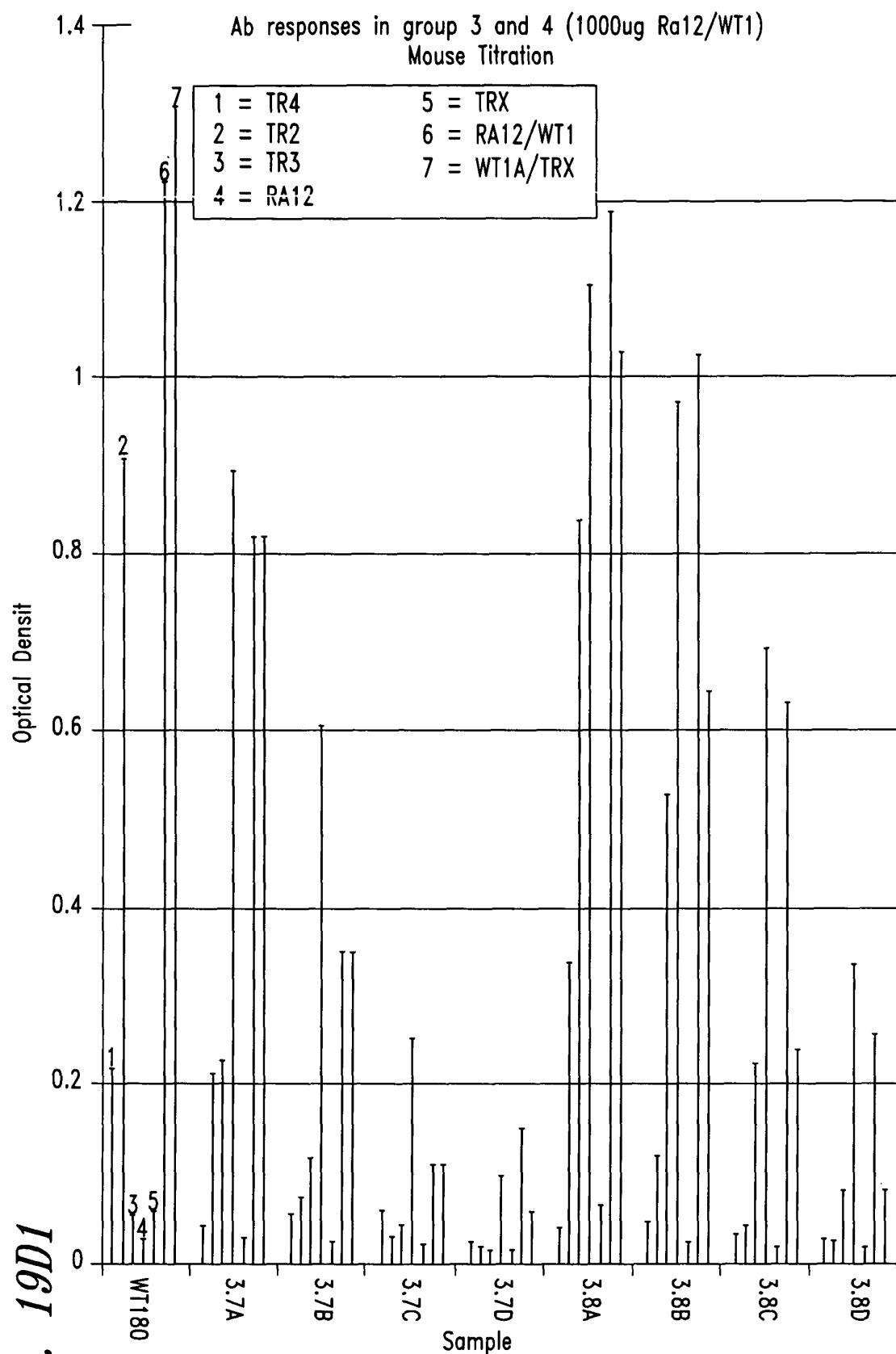


Fig. 19C3

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventor(s): Alexander Gaiger et al. Express Mail No. EV336590744US Docket No. 210121.465C12



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

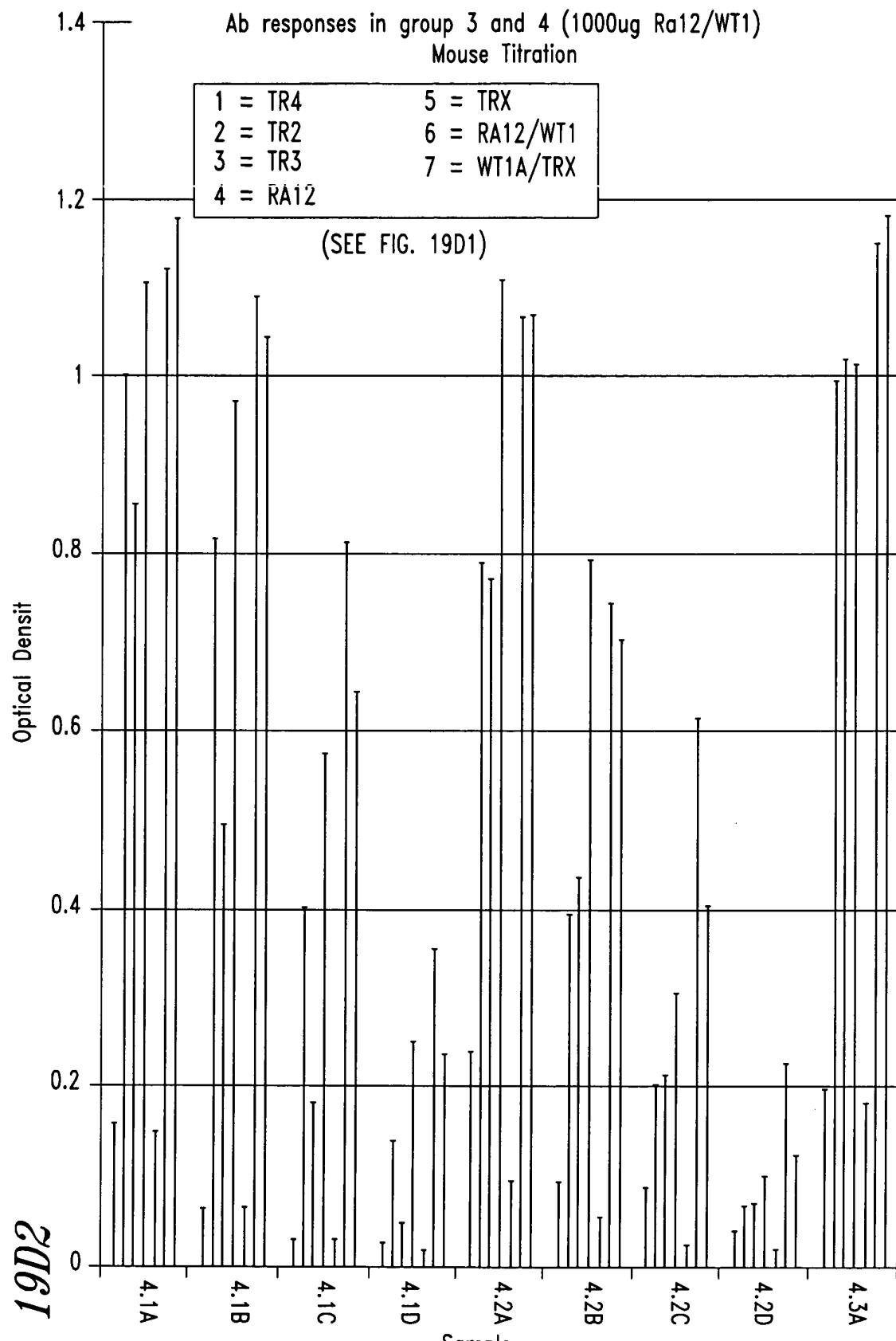


Fig. 19D2

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

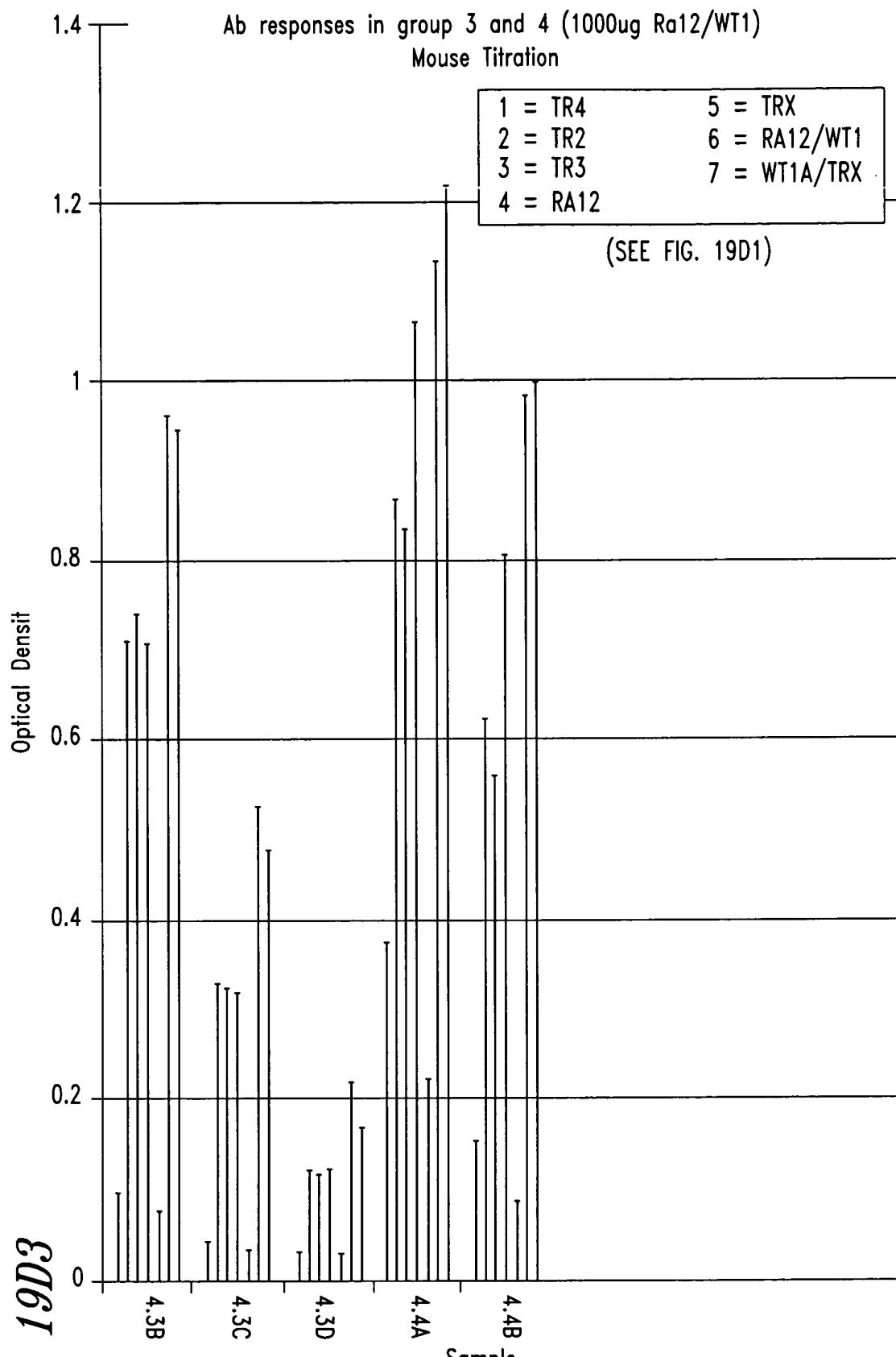


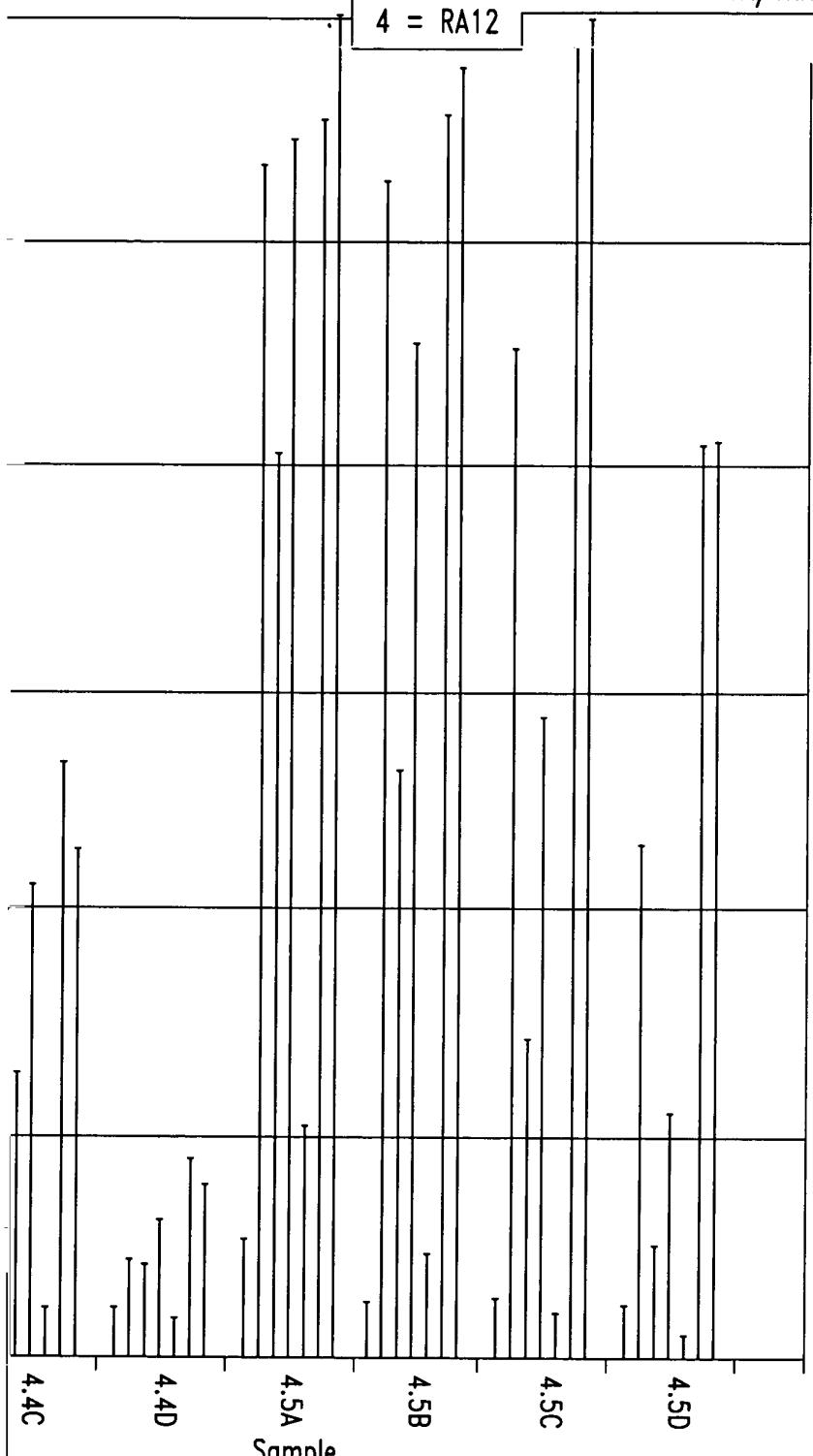
Fig. 19D3

WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

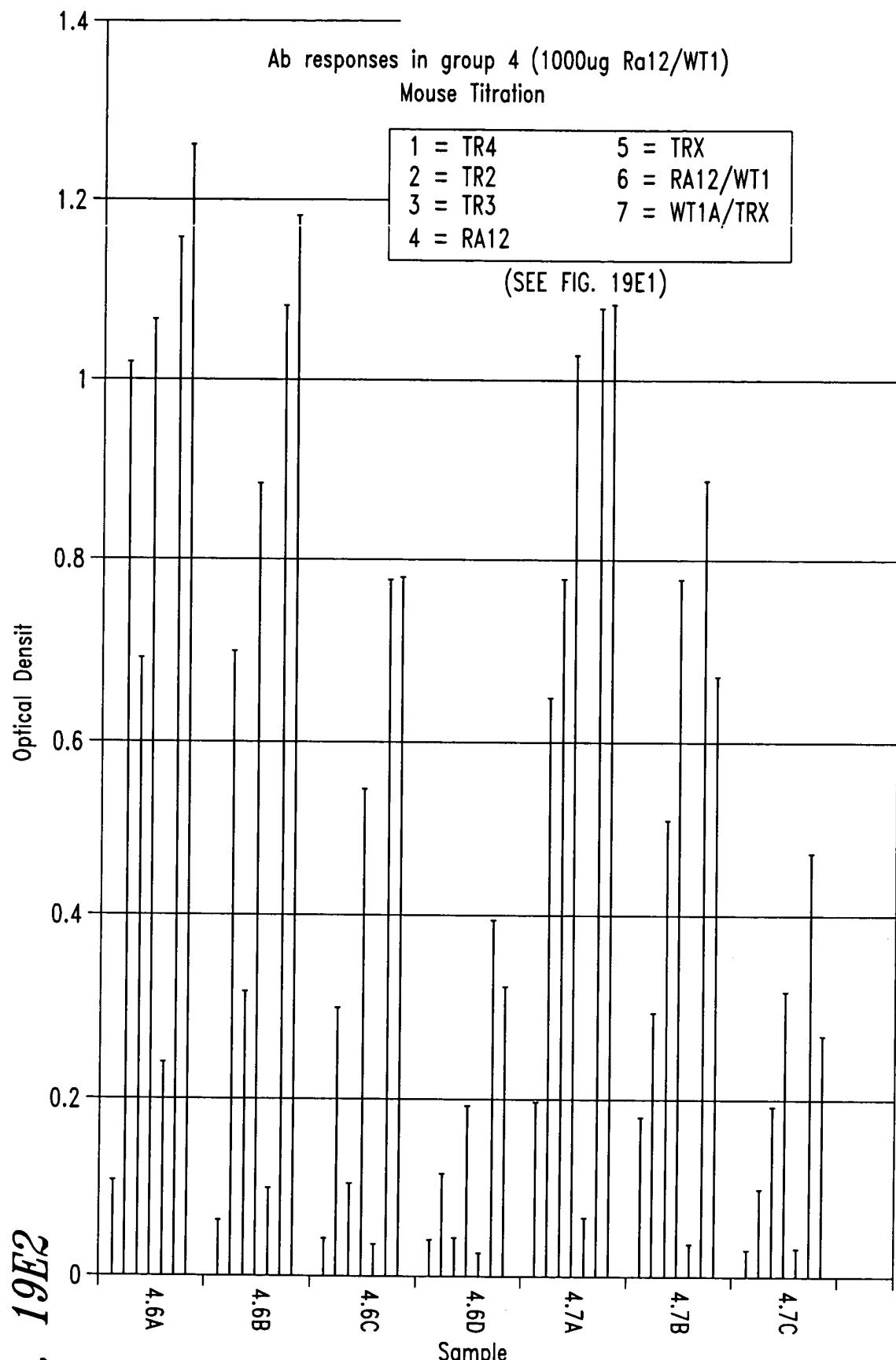
Ab responses in group 4 (1000ug Ra12/WT1)

Mouse Titration

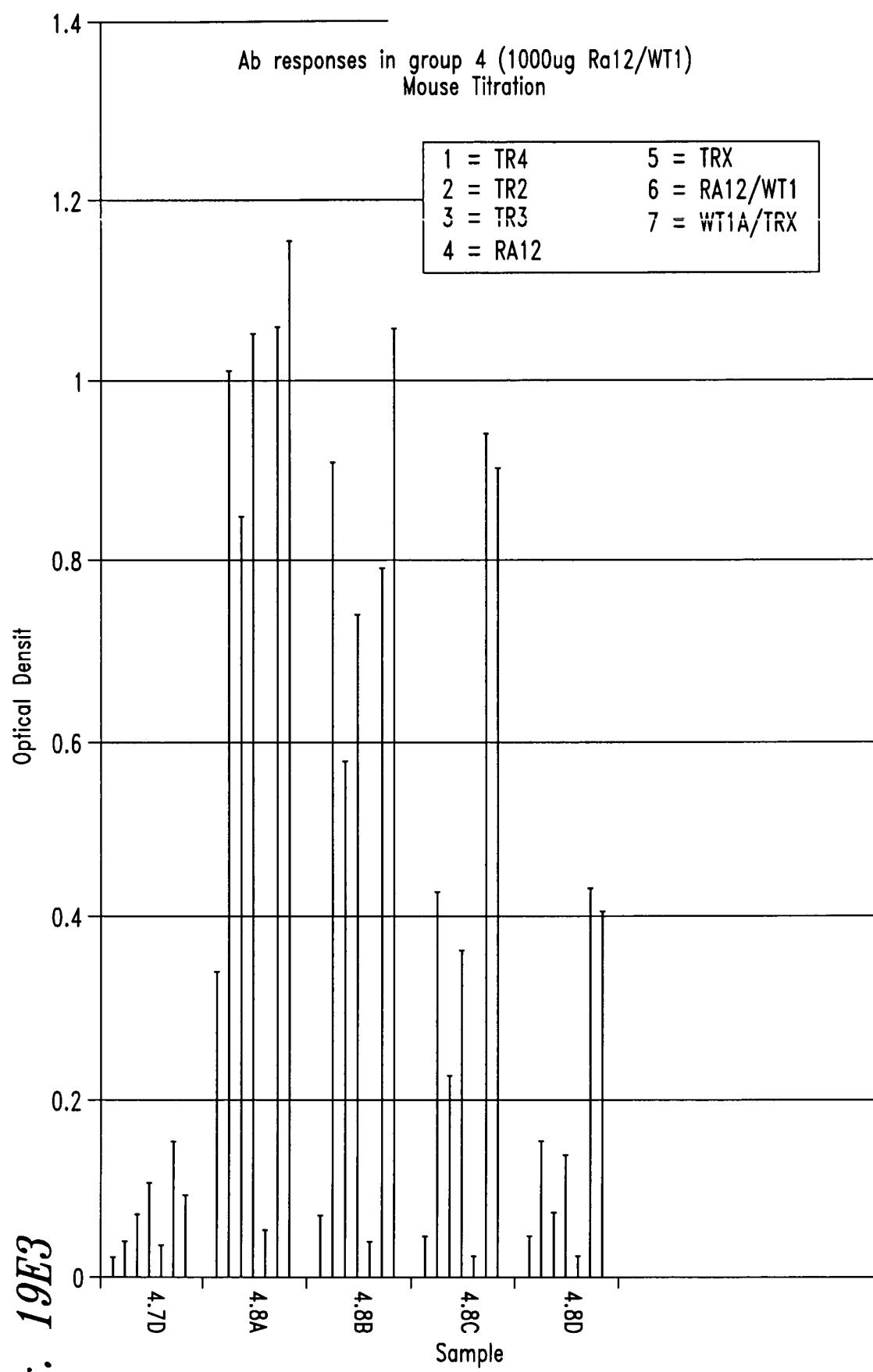
1 = TR4	5 = TRX
2 = TR2	6 = RA12/WT1
3 = TR3	7 = WT1A/TRX
4 = RA12	



Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE.
A: 1:500 Dilution, B: 1:2000, C: 1:8000, D: 1:16000

Proliferative T-cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 3x in vivo, after 2IVS)

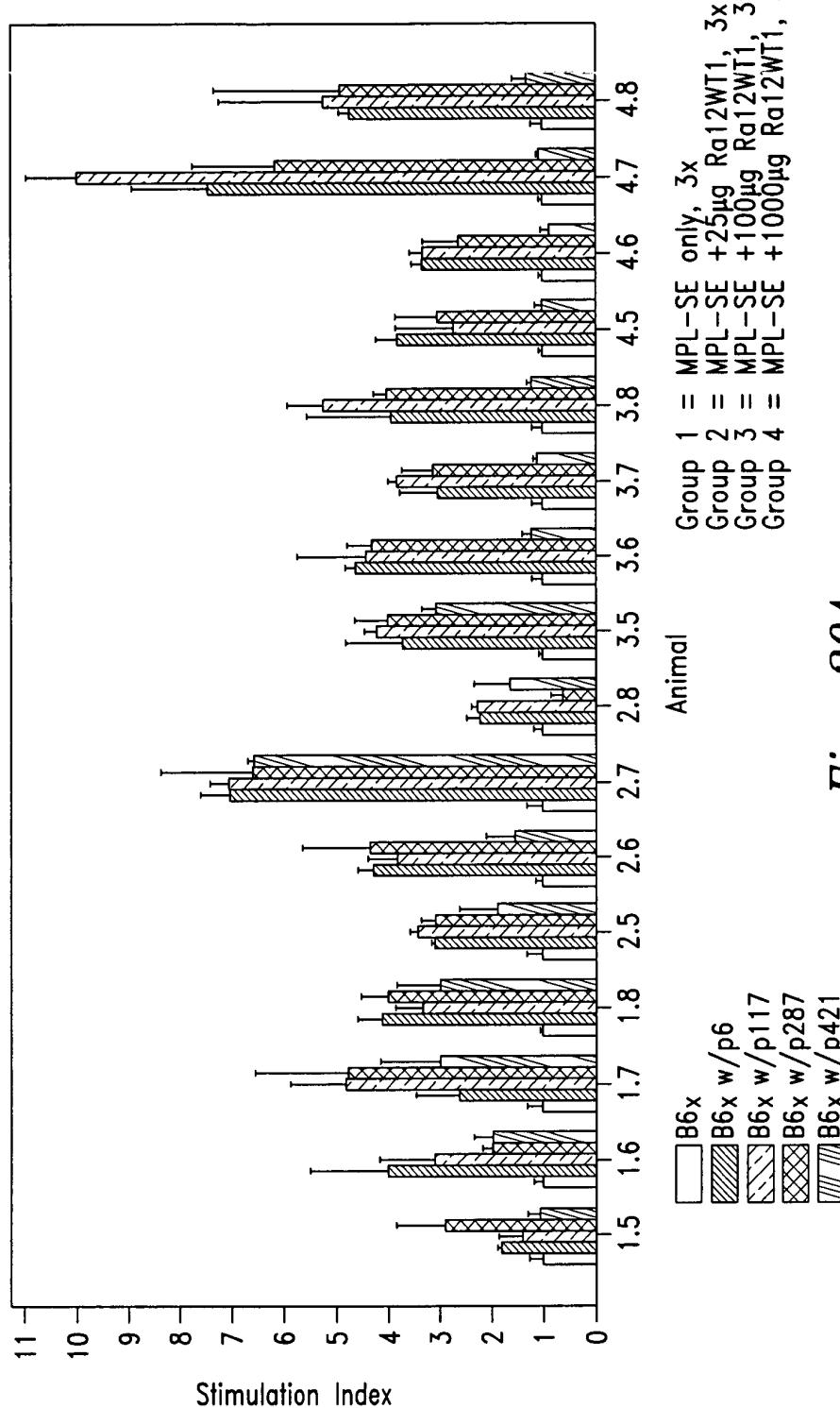
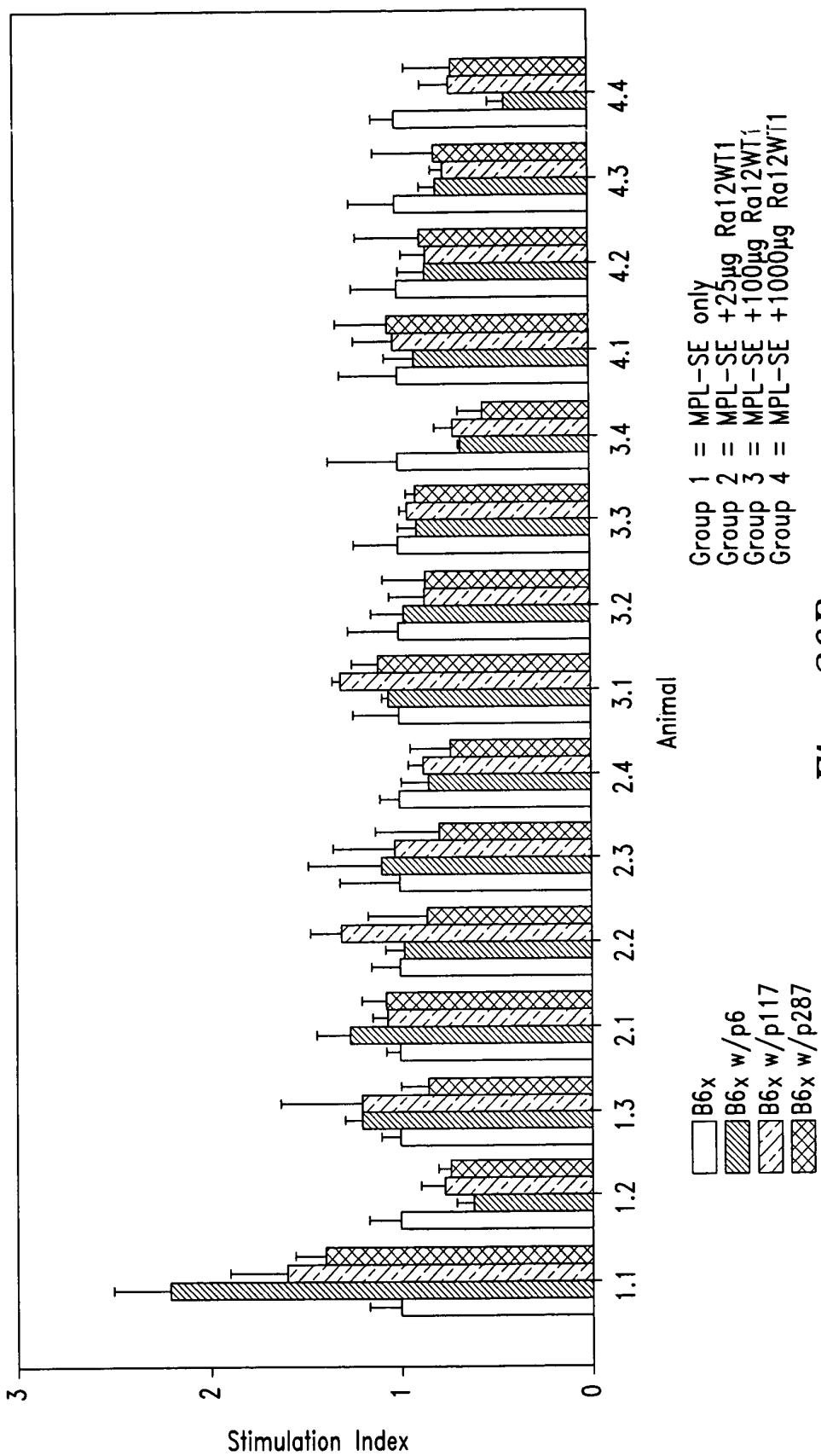


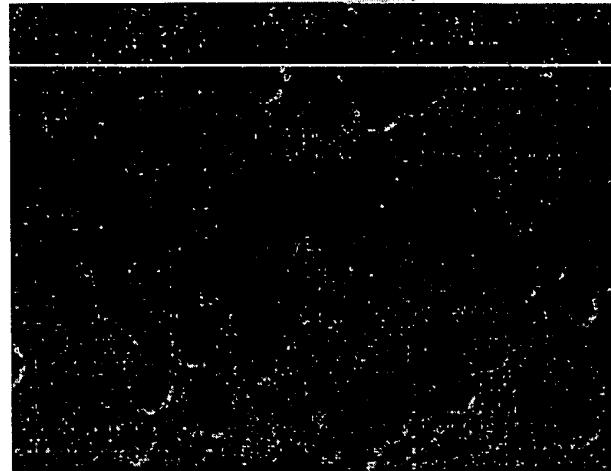
Fig. 20A

Proliferative T-cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 6x in vivo, after 2IVS)

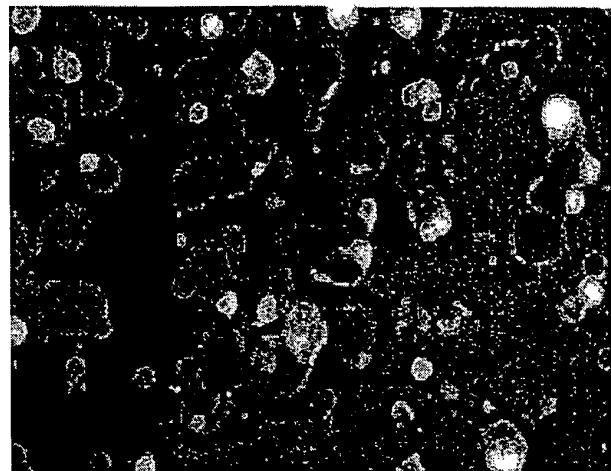


WT1 expression in human DC following adeno
WT1 and Vaccinia WT1 infection

Control
(uninfected human DC)



Adeno WT1
(infected human DC)



Vaccinia WT1
(infected human DC)

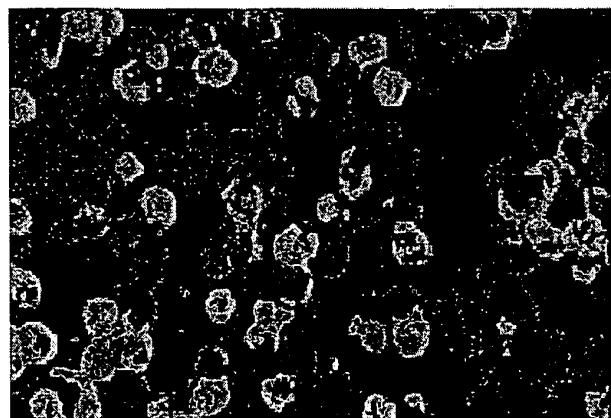
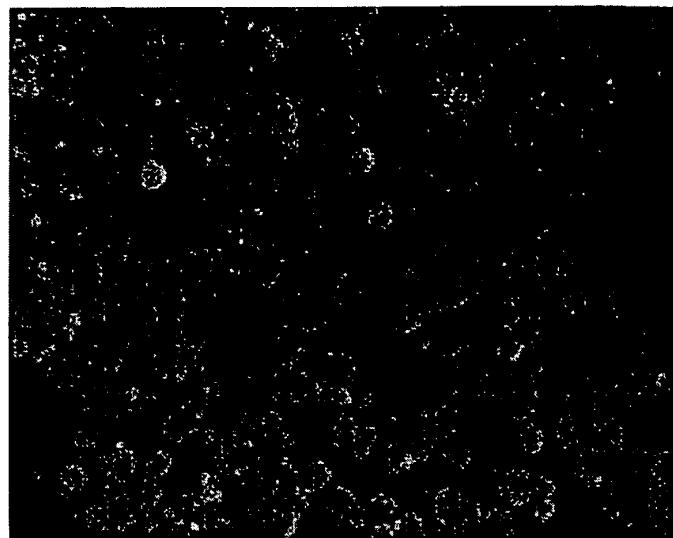


Fig. 21

WT1 can be expressed reproducible in human DC
following adeno WT1 infection and is not
induced by a control Adeno infection

Control
(Adeno EGFP
infected human DC)



Vaccinia WT1
(infected human
DC)

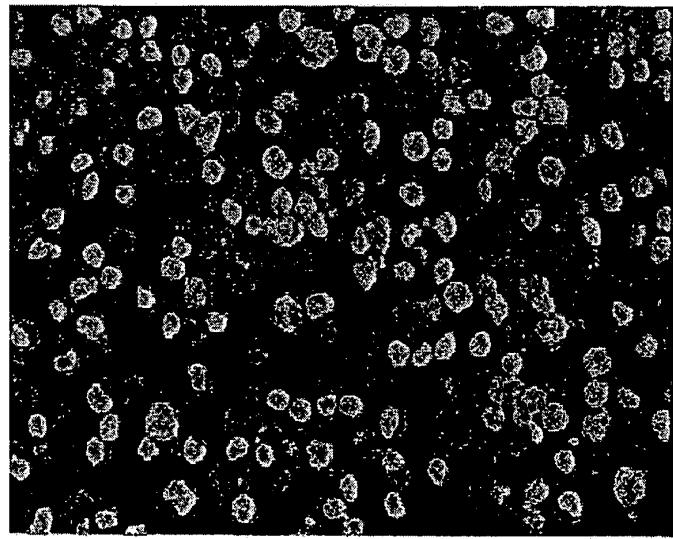


Fig. 22

WT1 whole gene in vitro priming elicits WT1 specific
T-cell responses (IFN-gamma ELISOT)

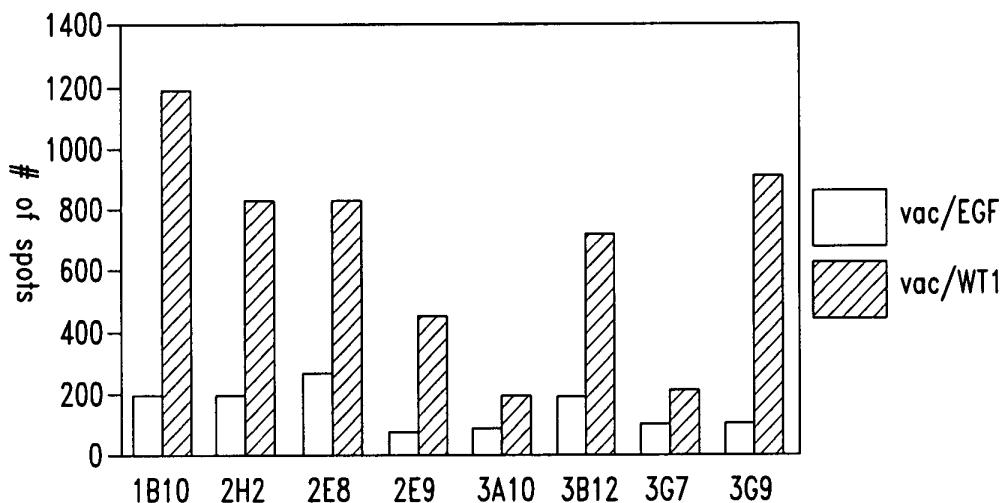


Fig. 23

WT-1 F cDNA 2-281

GGCTCCGACGTTGGGACCTGAACGCACTGCTGCCGGCAGTCCGTCCTGGTGGTGGTGGTGCAGTGCCTGCGACTGCCGTTAGCGGTGCAGCACAGTGGGCTCCGGTCTGGACTTCGACCGCCGGTGATCCGCATACGGTCCCTGGTGGTCCGGCACCGCCGCCGGCAGCGCCGCCGCCGCCGCCGCCGCCGCAGCTTCACTTCTCCGGCCAGTTCACTGGCACAGCCGGAGCCTGCGTACGGGCCCTTCGGTCCTCCCGCCAGCCAGGGCTCATCCGGCCAGGGCAGGATGTTCTAACCGCCCTACCTGCACAGCTGCCTCGAGAGGCCAGCCGCTATTGCAATCAGGGTACAGCACGGTCACCTCGACGGGACGCCAGCTACGGTCACACGCCCTCGCACCAGTCGGCAGTTCCCAACCACTATTCAAGCATGAGGATCCATGGGCCAGCAGGGCTCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCGGTATGGCTGCCACACCCCCACCGACAGCTGCACCGCAGCCAGGCTTGCTGAGGACGCCCTACAGCAGTGACAATTATACCAATGACATCCAGCTTGAATGCATGACCTGGAATCAGATGAACCTAGGAGCACCTAAAGGGCACAGCACAGGGTACGAGAGCGATAACCACACAACGCCATCCTCGGGAGCCCATACAGAATACACACGACGGTGCTTCAGAGGATTCACTGA

WT-1 F amino acid 2-281

GSDVRDLNALLPAVPSLGGGGGCALPVSQAAQWAPVLDFAAPPASAYGSLGPAPPAPPAPPAPPAPPHSFIKQEPWSGGAEPHEEQCLSAFTVHFSGQFTGAGACRYGPFGPPPPSQASSQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPSYGHTPSHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVGCHPTDSCTGSQALLRTPYSSDNLYQMTSQLECMTWNQMLGATLKGHSTGYESDNHTPILCGAQYRIHTHGVFRGIQ